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# Gastroprotective and cytotoxic effect of semisynthetic ferruginol derivatives

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## Abstract

The gastroprotective abietane diterpene ferruginol has been shown to present high cytotoxicity. In order to obtain active compounds with less cytotoxicity, 18 semisynthetic ferruginol derivatives and totarol were assessed for their gastroprotective effects in the HCl/ethanol-induced gastric lesion model in mice, as well as for cytotoxicity in human gastric epithelial cells (AGS) and human lung fibroblasts (MRC-5). At 20 mg kg$^{-1}$, the greatest gastroprotective effects were provided by abieta-8,11,13-triene (**1**), abieta-8,11,13-trien-12-yl-2-chloropropanoate (**8**), abieta-8,11,13-trien-12-yl propenoate (**9**), 12-(2,3,4,6-tetra-*O*-acetyl-$\beta$-D-glucopyranosyloxy)-abieta-8,11,13-triene (**17**) and 12-($\beta$-D-galactopyranosyloxy)-abieta-8,11,13-triene (**18**), all of which were as active as the reference drug lansoprazole at 20 mg kg$^{-1}$, reducing gastric lesions by 69, 76, 67, 72 and 61%, respectively. No relation was observed between lipophilicity and the gastroprotective effect. Compounds that showed the greatest cytotoxicity towards AGS cells were ferruginol (**2**), the corresponding formate (**5**), acetate (**6**), propionate (**7**), **8**, **9**, 12-($\beta$-D-glucopyranosyloxy)-abieta-8,11,13-triene (**16**), **18** and totarol (**20**) (IC50 18–44 $\mu$M). Ferruginol and compounds **5**–**9**, **16**, **18** and **20** were the most toxic compounds against fibroblasts (IC50 19–56 $\mu$M), with a correlation to AGS cells. The derivative **19** was much more active against AGS cells than towards fibroblasts. The best activity/cytotoxicity ratio was found for compound **17**, with a lesion index comparable with lansoprazole at 20 mg kg$^{-1}$ and cytotoxicity >1000 $\mu$M towards MRC-5 and AGS cells, respectively. In conclusion, some derivatives showed a better gastroprotective effect/cytotoxicity ratio than the parent compound ferruginol. A total of 13 new compounds are reported here for the first time.

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## Introduction

Gastric lesions affect nearly 5% of the global population. Gastric ulcers develop when the balance between aggressive and defensive factors in the stomach is lost. The aggressive factors may be endogenous or exogenous. Endogenous factors include hydrochloric acid, bile, pepsin, platelet-activating factor, endothelins, leukotrienes and reactive oxygen species. Exogenous factors include non-steroidal anti-inflammatory drugs, nicotine, stress, tension and *Helicobacter pylori*. Defensive factors include mucus, bicarbonate, mucosal blood flow, motility, cellular regeneration, prostaglandins, nitric oxide, epidermal growth factor, antioxidants and antioxidative enzymes.

Drug treatment of peptic ulcers is targeted at either counteracting aggressive factors or stimulating the gastric mucosa defence. In addition to progress in chemistry and pharmacology in the production of new drugs, the plant kingdom is also a useful source of new anti-ulcer compounds for development. Some terpenes isolated from higher plants and several of their derivatives show preventive activity against gastric lesions induced by different ulcerogenic procedures in animals (Hiruma-Lima et al 2001; Ann et al 2002; Schmeda-Hirschmann et al 2002, 2005; Arrieta et al 2003; Silva Melo et al 2003; Favier et al 2005; Reyes et al 2005; Rodríguez et al 2005).

Dehydroabietic acid derivatives have been shown to display gastroprotective effects in animal models. Ferruginol is an abietane diterpene occurring in the wood and bark of the gymnosperm *Prumnopitys andina* (Poepp. Ex Endl.) de Laub and *Podocarpus nubigena* Lind. (Podocarpaceae), both native species of Chile. A dose–response study of ferruginol in

the HCl/ethanol-induced gastric lesion model in mice showed that ferruginol inhibited gastric lesions at 25 mg $kg^{-1}$, similar to lansoprazole at 20 mg $kg^{-1}$ (Rodríguez et al 2006). However, the effects of totarol and ferruginol derivatives on the prevention of gastric lesions in animal models has been not reported to date.

To reveal some structure–activity trends/relationships for this diterpene, some 18 semisynthetic ferruginol derivatives were prepared and assessed for gastroprotective effects against experimentally induced gastric lesions in mice and for cytotoxicity in human lung fibroblasts (MRC-5) and human epithelial gastric (AGS) cells. The closely related compound totarol, which also occurs in the plants used as a source of ferruginol, was included in the study.

## Materials and Methods

### Isolation of ferruginol

The wood of *P. nubigena* Lind. and stem bark of *P. andina* (Poepp. ex Endl.) de Laub. (Podocarpaceae) were collected in Oncol (Valdivia, IX Region, Chile) in July 2004. The wood and bark were chopped, dried at 40°C and powdered. The plants were identified by Patricio Peñaillillo, Departamento de Botánica, Universidad de Talca, Chile, and voucher specimens (nos 3008 and 3009, respectively) were deposited at the Herbarium of the Universidad de Talca.

The powdered stem bark of *P. andina* (3 kg) was exhaustively extracted under reflux with petroleum ether (2×4 L each). The combined extracts were filtered and evaporated to dryness under reduced pressure, affording 120 g of solubles. The extract was chromatographed on a silica gel G 60 column (200–500 $\mu$m; Merck) using a petroleum ether/EtOAc (PE-EtOAc) gradient system (1:0 to 0:1). The ferruginol-containing fractions eluted with PE-EtOAc 8:2 and 7:3. The crude ferruginol was further purified by column chromatography with a petroleum ether/diethyl ether gradient to afford, after repeated crystallization in cold *n*-hexane, some 7.5 g of ferruginol (compound **2**). The stem wood of *P. nubigena* Lind. (1.28 kg) was worked-up under the same chromatographic conditions as above affording, after repeated recrystallization, some 120 mg of totarol.

### Isolation of ferruginol derivatives

Melting points were determined on a Koffler hot-stage apparatus (Electrothermal 9100) and were uncorrected. Optical rotations were obtained for solutions in $CHCl_3$ or MeOH (concentrations expressed in g/100 mL) on a Jasco DIP 370 polarimeter. Infrared spectra were recorded on a Nicolet Nexus 470 Fourier transform infrared instrument. $^1$H NMR spectra were recorded at 400 MHz and $^{13}$C NMR data were obtained at 100 MHz on a Bruker spectrometer ($\delta$ scale). Spectral assignments were made with the assistance of DEPT and HMBC correlation spectra. $CDCl_3$ and $CD_3OD$ were used as solvents. Mass spectra were recorded on a Varian spectrometer at 70 eV and are presented as m/z (% rel. int.).

Thin-layer chromatography spots were visualized by spraying the chromatograms with *p*-anisaldehyde/acetic acid/$H_2SO_4$/ethanol (2:20:10:170) and heating at 110°C for 3 min. Column chromatography was performed over a Merck Kieselgel 60, particle size 0.063–0.200 mm. All solvents were dried and purified before use according to standard procedures. When required, reactions were carried out under an inert dry nitrogen atmosphere. The compounds were prepared as follows.

*Compound **1***

In a two-necked flask containing 430 mg (1.027 mmol) of ferruginol triflate (compound **4**) in methanol (20 mL) under $H_2$ atmosphere, 150 mg of Pd/C 10% and 3 mL triethylamine were added. The resulting solution was stirred for 24 h. After dilution with cold water (50 mL), the solution was extracted with $CH_2Cl_2$ (2×50 mL). The organic phase was successively washed with aqueous HCl (2×50 mL, 1 N) and saturated $NaHCO_3$ solution (2×50 mL), dried over $MgSO_4$ (2 g) and concentrated in-vacuo. Purification by column chromatography on silica gel using a PE-EtOAc 100:0 to 97:3 gradient gave compound **1** (182 mg, 66%).

*Compound **3***

In a flask containing a stirred suspension of 300 mg (1.05 mmol) of ferruginol in dimethylformamide (5 mL) and NaH (60%, 50 mg, 1.25 mmol) at 0°C under $N_2$ atmosphere, dimethyl sulfate (0.5 mL, 5.23 mmol) was added dropwise. The resulting suspension was stirred for 24 h. After dilution with cold water (50 mL), the solution was extracted with $CH_2Cl_2$ (2×50 mL). The organic phase was successively washed with aqueous HCl (2×50 mL, 1 N) and saturated $NaHCO_3$ solution (2×50 mL), dried over $MgSO_4$ (2 g) and concentrated in-vacuo. Purification by column chromatography on silica gel using a PE-EtOAc 100:0 to 97:3 gradient gave compound **3** (229 mg; 73%).

*Compound **4***

In a flask containing a stirred solution of 900 mg (3.14 mmol) of ferruginol and triethylamine (1 mL) in $CH_2Cl_2$ (20 mL) at 0°C under $N_2$ atmosphere, 1 mL (5.94 mmol) of trifluoromethanesulfonic anhydride was added dropwise. The resulting suspension was stirred for 18 h. After the addition of 50 mL $CH_2Cl_2$, the organic phase was successively washed with aqueous HCl (2×50 mL, 1 N), 2% NaCl solution (2×50 mL) and concentrated in-vacuo. Purification by column chromatography on silica gel using a PE-EtOAc 100:0 to 97:3 gradient gave compound **4** (1.1 g; 85%).

*Compound **5***

In a two-necked flask containing ferruginol (200 mg, 0.698 mmol) dissolved in 10 mL of $CH_2Cl_2$ under $N_2$ atmosphere, 1 mL formic acid was added dropwise. The resulting solution was stirred for 4 days, neutralized with sodium carbonate to pH 7, extracted with $CH_2Cl_2$ (2×50 mL) and concentrated in-vacuo. Purification by column chromatography on silica gel using a PE-EtOAc 100:0 to 97:3 gradient gave compound **5** (10 mg, 5%).

*Compound **6***

Acetylchloride (4 mL) was added to a stirred solution of ferruginol (650 mg, 2.27 mmol) in pyridine (4 mL) at 0°C under

$N_2$ atmosphere. The resulting solution was stirred for 24 h. After dilution with cold water (50 mL), the solution was extracted with EtOAc (2×50 mL). The organic phase was successively washed with aqueous HCl (2×100 mL, 1 N) and saturated $NaHCO_3$ solution (2×50 mL), dried over $MgSO_4$ (2 g) and concentrated in-vacuo. Purification by column chromatography on silica gel using a PE-EtOAc 100:0 to 95:5 gradient gave compound **6** (719 mg; 97%).

*Compound **7***

Propanoylchloride (0.50 mL) was added to a stirred solution of ferruginol (218 mg, 0.761 mmol) in pyridine (1 mL) at 0°C under $N_2$ atmosphere. The resulting solution was stirred for 24 h. After dilution with cold water (50 mL), the solution was extracted with $CH_2Cl_2$ (2×50 mL). The organic phase was successively washed with aqueous HCl (2×100 mL, 1 N) and saturated $NaHCO_3$ solution (2×50 mL), dried over $MgSO_4$ (1 g) and concentrated in-vacuo. Purification by column chromatography on silica gel using a PE-EtOAc 100:0 to 95:5 gradient gave compound **7** (126 mg; 53%).

*Compound **8***

2-Chloropropanoylchloride (0.50 mL) was added to a stirred solution of ferruginol (200 mg, 0.698 mmol) in pyridine (1 mL) at 0°C under $N_2$ atmosphere. The resulting solution was stirred for 24 h. After dilution with cold water (50 mL), the solution was extracted with $CH_2Cl_2$ (2×50 mL). The organic phase was successively washed with aqueous HCl (2×100 mL, 1 N) and saturated $NaHCO_3$ solution (2×50 mL), dried over $MgSO_4$ (1 g) and concentrated in-vacuo. Purification by column chromatography on silica gel using a PE-EtOAc 100:0 to 95:5 gradient gave compound **8** (206 mg; 73%).

*Compound **9***

3-Chloropropanoylchloride (0.50 mL) was added to a stirred solution of ferruginol (150 mg, 0.524 mmol) in triethylamine (3 mL) at 0°C under $N_2$ atmosphere. The resulting solution was stirred for 48 h. After dilution with cold water (50 mL), the solution was extracted with $CH_2Cl_2$ (2×50 mL). The organic phase was successively washed with aqueous HCl (2×100 mL, 1 N) and saturated $NaHCO_3$ solution (2×50 mL), dried over $MgSO_4$ (1 g) and concentrated in-vacuo. Purification by column chromatography on silica gel using a PE-EtOAc 100:0 to 95:5 gradient gave compound **9** (30 mg; 17%). In the synthesis of compound **9**, the reaction proceeds with concomitant dehydrochlorination.

*Compound **10***

Benzoylchloride (0.50 mL) was added to a stirred solution of ferruginol (200 mg, 0.698 mmol) in pyridine (1 mL) at 0°C under $N_2$ atmosphere. The resulting solution was stirred for 12 h. After dilution with cold water (50 mL), the solution was extracted with $CH_2Cl_2$ (2×50 mL). The organic phase was successively washed with aqueous HCl (2×50 mL, 1 N) and saturated $NaHCO_3$ solution (2×50 mL), dried over $MgSO_4$ (2 g) and concentrated in-vacuo. Purification by column chromatography on silica gel using a PE-EtOAc 100:0 to 95:5 gradient gave compound **10** (100 mg; 37%).

*Compound **11***

2-Methylbenzoylchloride (0.50 mL) was added to a stirred solution of ferruginol (200 mg, 0.698 mmol) in pyridine (1 mL) at 0°C under $N_2$ atmosphere. The resulting solution was stirred for 12 h. After dilution with cold water (50 mL), the solution was extracted with $CH_2Cl_2$ (2×50 mL). The organic phase was successively washed with aqueous HCl (2×50 mL, 1 N) and saturated $NaHCO_3$ solution (2×50 mL), dried over $MgSO_4$ (2 g) and concentrated in-vacuo. Purification by column chromatography on silica gel using a PE-EtOAc 100:0 to 95:5 gradient gave compound **11** (158 mg; 56%).

*Compound **12***

4-Methylbenzoylchloride (0.50 mL) was added to a stirred solution of ferruginol (200 mg, 0.698 mmol) in pyridine (1 mL) at 0°C under $N_2$ atmosphere. The resulting solution was stirred for 12 h. After dilution with cold water (50 mL), the solution was extracted with $CH_2Cl_2$ (2×50 mL). The organic phase was successively washed with aqueous HCl (2×50 mL, 1 N) and saturated $NaHCO_3$ solution (2×50 mL), dried over $MgSO_4$ (2 g) and concentrated in-vacuo. Purification by column chromatography on silica gel using a PE-EtOAc 100:0 to 95:5 gradient gave compound **12** (246 mg; 87%).

*Compound **13***

4-Methoxybenzoylchloride (0.50 mL) was added to a stirred solution of ferruginol (200 mg, 0.698 mmol) in pyridine (1 mL) at 0°C under $N_2$ atmosphere. The resulting solution was stirred for 12 h. After dilution with cold water (50 mL), the solution was extracted with $CH_2Cl_2$ (2×50 mL). The organic phase was successively washed with aqueous HCl (2×50 mL, 1 N) and saturated $NaHCO_3$ solution (2×50 mL), dried over $MgSO_4$ (2 g) and concentrated in-vacuo. Purification by column chromatography on silica gel using a PE-EtOAc 100:0 to 95:5 gradient gave compound **13** (251 mg; 78%).

*Compound **14***

3-Nitrobenzoylchloride (100 mg) was added to a stirred solution of ferruginol (150 mg, 0.524 mmol) in pyridine (1 mL) at 0°C under $N_2$ atmosphere. The resulting solution was stirred for 12 h. After dilution with cold water (50 mL), the solution was extracted with $CH_2Cl_2$ (2×50 mL). The organic phase was successively washed with aqueous HCl (2×50 mL, 1 N) and saturated $NaHCO_3$ solution (2×50 mL), dried over $MgSO_4$ (2 g) and concentrated in-vacuo. Purification by column chromatography on silica gel using a PE-EtOAc 100:0 to 95:5 gradient gave compound **14** (200 mg; 88%).

*Compound **15***

4-Nitrobenzoylchloride (100 mg) was added to a stirred solution of ferruginol (150 mg, 0.524 mmol) in pyridine (1 mL) at 0°C under $N_2$ atmosphere. The resulting solution was stirred for 12 h. After dilution with cold water (50 mL), the solution was extracted with $CH_2Cl_2$ (2×50 mL). The organic phase was successively washed with aqueous HCl (2×50 mL, 1 N) and saturated $NaHCO_3$ solution (2×50 mL), dried over

$MgSO_4$ (2 g) and concentrated in-vacuo. Purification by column chromatography on silica gel using a PE-EtOAc 100:0 to 95:5 gradient gave compound **15** (148 mg; 65%).

*Compound **17***

A solution of tetra-*O*-acetyl-α-D-glucopyranosyl bromide (450 mg, 1.09 mmol) in hexane (5 mL) was added dropwise to a stirred suspension of ferruginol (300 mg, 1.05 mmol) and activated sieves (3 Å, 0.5 g) in hexane under $N_2$ atmosphere in a flask masked from the light. After 3 h, silver triflate (290 mg, 1.12 mmol) was added. The mixture was stirred for 14 h, filtered and concentrated under reduced pressure. After column chromatography on silica gel using a PE-EtOAc 100:0 to 70:30 gradient, compound **17** was obtained (378 mg; w/w yield 58%).

*Compound **16***

The tetraacetate **17** (300 mg, 0.486 mmol) in MeOH (5 mL) at room temperature under $N_2$ atmosphere was treated with NaH (100 mg, 60%). The solution was stirred for 14 h, filtered and concentrated under reduced pressure. After permeation on Sephadex LH 20 with MeOH, compound **16** was obtained (210 mg; w/w yield 96%).

*Compound **19***

A solution of tetra-*O*-acetyl-α-D-galactopyranosyl bromide (480 mg, 1.16 mmol) in hexane (10 mL) was added dropwise to a stirred suspension of ferruginol (300 mg, 1.05 mmol) and activated sieves (3 Å, 1.0 g) in hexane under $N_2$ atmosphere in a flask masked from the light. After 3 h, silver triflate (310 mg, 1.20 mmol) was added. The mixture was stirred for 14 h, filtered and concentrated under reduced pressure. After column chromatography on silica gel using a PE-EtOAc 100:0 to 70:30 gradient, compound **19** was obtained (250 mg; w/w yield 39%).

*Compound **18***

The tetraacetate **19** (140 mg, 0.227 mmol) in MeOH (5 mL) at room temperature under $N_2$ atmosphere was treated with NaH (45 mg, 60%). The solution was stirred for 14 h, filtered and concentrated under reduced pressure. After permeation on Sephadex LH 20 with MeOH, compound **18** was obtained (100 mg; w/w yield 98%).

### Animals

Swiss albino mice, 30 ± 3 g, were purchased from the Instituto de Salud Publica de Chile, Santiago, Chile. The animals were fed on certified Champion diet with free access to water under standard conditions: 12-h dark–light cycle, 50% relative humidity and 22°C room temperature. The animals were fasted for 24 h before the ulcerogenic assays and the reference compound (lansoprazole), the diterpene ferruginol, its derivatives and totarol were administered orally.

### HCl/ethanol-induced lesions

The gastroprotective activity of the compounds was assessed in the HCl/ethanol-induced lesion model as described by Rodríguez et al (2005) and Schmeda-Hirschmann et al (2005). The protocols were approved by the Universidad de Talca Institutional Animal Care and Use Committee, which follows the recommendations of the Canadian Council on Animal Care (Olfert et al 1993).

Mice were randomly distributed into groups of seven or eight animals each and fasted for 24 h with free access to water before the experiment. For comparison of the ferruginol derivatives and totarol, a single oral dose of 20 mg $kg^{-1}$ was selected because in a previous experiment we determined that the lesion index was reduced by about 50% by ferruginol at 20 mg $kg^{-1}$. Compounds were suspended in a 12% solution of the non-ionic detergent Tween 80. At 50 min after oral administration of the compounds, lansoprazole (2-[[[3-methyl-4-(2,2,2-trifluroethoxy)-2-pyridyl]methyl]sulfinyl] benzimidazole) (20 mg $kg^{-1}$) or 12% Tween 80 (10 mL $kg^{-1}$), all groups were treated orally with 0.2 mL of a solution containing 0.3 M HCl/60% ethanol (HCl/ethanol) to induce gastric lesions. Animals were killed 1 h after the administration of HCl/ethanol, 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 HCl/ethanol-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. The percentage gastroprotective effect was calculated as follows:

$$\%\ \text{lesion reduction} = 1 - \left( \frac{\text{lesion index of sample}}{\text{lesion index of control}} \right) \times 100$$

### 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 Eagle's minimum essential medium, with Earle's salts, 2 mM L-glutamine and 2.2 g $L^{-1}$ sodium bicarbonate, supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 IU $mL^{-1}$ penicillin and 100 μg $mL^{-1}$ streptomycin in a humidified incubator with 5% $CO_2$ in air at 37°C. 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 $L^{-1}$ sodium bicarbonate, supplemented with 10% heat-inactivated FBS, 100 IU $mL^{-1}$ penicillin and 100 μg $mL^{-1}$ 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 and AGS cells were treated with medium containing the diterpene derivatives as well as with the reference compound lansoprazole at concentrations ranging from 0 to 1000 $\mu$M. The products were first dissolved in dimethylsulfoxide and then in the corresponding culture medium supplemented with 2% FBS. The final content of dimethylsulfoxide 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 assay was carried out as described by Rodríguez & Haun (1999). To calculate the IC50 (concentration that produces a 50% inhibitory effect on the evaluated parameter), the results were transformed to percentage of controls and the IC50 values were graphically obtained from the dose–response curves.

### Lipophilicity

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

### Statistical analysis

Results were expressed as the mean ± s.d. In all experiments, statistical differences between several treatments and their respective control were determined by one-way analysis of variance 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 $P < 0.01$. All statistical analyses were performed using Statistica 5.1 software (StatSoft, Inc.) and Statistical Package S-Plus 2000.

## Results and Discussion

Some 18 ferruginol derivatives were prepared by simple chemical reactions. The diterpene totarol was isolated from the stem bark of *P. nubigena*. The chemical structures of the compounds are shown in Figure 1. The NMR spectral data of compounds **4**, **5**, **7**, **8** and **10**–**19** are presented in Tables 1–6.

Abieta-8,11,13-triene (compound **1**): colourless oil. HRMS: calculated for $C_{20}H_{30}$: 270.2348, found: 270.2345.

12-Hydroxyabieta-8,11,13-triene (ferruginol; compound **2**): white powder, mp 50–53°C. HRMS: calculated for $C_{20}H_{30}O$: 286.2297, found: 286.2295.

12-Methoxyabieta-8,11,13-triene (compound **3**): colourless oil. HRMS: calculated for $C_{21}H_{32}O$: 300.2453, found: 300.2450.

Abieta-8,11,13-trien-12-yl trifluoromethanesulfonate (ferruginol triflate; compound **4**): white powder, mp 65–68°C. HRMS: calculated for $C_{21}H_{29}O_3F_3S$: 418.1790, found: 418.1785. MS (EI): m/z (rel. int. %): 418 [$M^+$] (96), 403 (100), 375 (11), 361 (28), 347 (29), 333 (66), 321 (100), 307 (74), 285 (13), 201 (25), 143 (84), 83 (31), 69 (100). IR $\nu_{max}$: 2935, 1490, 1415,1210, 1141, 890, 860 $cm^{-1}$. $[\alpha]_D^{20}$: +36 ($CHCl_3$;c = 0.5).

Abieta-8,11,13-trien-12-yl formate. (compound **5**): pale yellow powder, mp 77–79°C. HRMS: calculated for $C_{21}H_{30}O_2$: 314.2246, found: 314.2250. MS (EI): m/z (rel. int. %): 314 [$M^+$] (25), 299 (51), 243 (9), 229 (33), 217 (85), 203 (41), 189 (22), 175 (30), 128 (17), 91 (15), 69 (100). IR $\nu_{max}$: 2925, 1766, 1653, 1500, 1134, 902 $cm^{-1}$. $[\alpha]_D^{20}$: +62 ($CHCl_3$;c = 0.5).

Abieta-8,11,13-trien-12-yl acetate (compound **6**): white solid, mp 61–63°C. HRMS: calculated for $C_{22}H_{32}O_2$: 328.2402, found: 328.2410.

Abieta-8,11,13-trien-12-yl propionate (compound **7**): colourless oil. HRMS: calculated for $C_{23}H_{34}O_2$: 342.2559, found: 342.2554. MS (EI): m/z (rel. int. %): 342 [$M^+$] (21), 286 (100), 271 (45), 229 (5), 189 (13), 175 (14), 147 (9), 69 (9), 57 (5). IR $\nu_{max}$: 2961, 1757, 1495, 1462, 1164, 1146, 903 $cm^{-1}$. $[\alpha]_D^{20}$: +46 ($CHCl_3$;c = 0.5).

Abieta-8,11,13-trien-12-yl-2-chloropropanoate (compound **8**): yellow oil. HRMS: calculated for $C_{23}H_{33}O_2Cl$: 376.2169, found: 376.2178. MS (EI): m/z (rel. int. %): 378 [$M^+ + 2$] (28), 376 [$M^+$] (80), 361 (100), 333 (6), 319 (11), 305 (8), 291 (35), 286 (69), 279 (65), 271 (38), 265 (30), 251 (12), 229 (12), 215 (8), 201 (22), 175 (25), 147 (28), 69 (38). IR $\nu_{max}$: 2926, 1766, 1494, 1243, 1164, 907 $cm^{-1}$. $[\alpha]_D^{20}$: +53 ($CHCl_3$;c = 0.49).

Abieta-8,11,13-trien-12-yl propenoate (compound **9**): pale yellow solid, mp 60–63°C. HRMS: calculated for $C_{23}H_{32}O_2$: 340.2402, found: 340.2400.

Abieta-8,11,13-trien-12-yl-benzoate (compound **10**): white solid, mp 136–138°C. HRMS: calculated for $C_{27}H_{34}O_2$: 390.2559, found: 390.2551. MS (EI): m/z (rel. int. %): 390 [$M^+$] (40), 375 (18), 285 (28), 147 (4), 105 (100), 77(13), 69 (3). IR $\nu_{max}$: 2929, 1729, 1599, 1494, 1450, 1242, 713 $cm^{-1}$. $[\alpha]_D^{20}$: +42 ($CHCl_3$;c = 0.5).

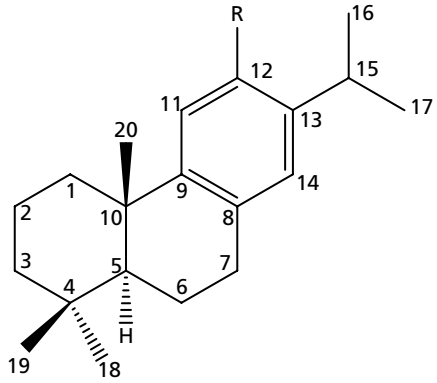
Abieta-8,11,13-trien-12-yl-2′-methylbenzoate (compound **11**): white solid, mp 70–73°C. HRMS: calculated for $C_{28}H_{36}O_2$: 404.2715, found: 404.2700. MS (EI): m/z (rel. int. %): 404 [$M^+$] (33), 389 (3), 285 (5), 147 (5), 119 (100), 105 (3), 91 (23), 69 (3). IR $\nu_{max}$: 2921, 1738, 1494, 1460, 1239, 896, 738 $cm^{-1}$. $[\alpha]_D^{20}$: +54 ($CHCl_3$;c = 0.5).

Abieta-8,11,13-trien-12-yl-4′-methylbenzoate (compound **12**): white solid, mp 132–135°C. HRMS: calculated for $C_{28}H_{36}O_2$: 404.2715, found: 404.2702. IR $\nu_{max}$: 2928, 1728, 1611, 1494, 1270, 1245, 900, 750 $cm^{-1}$. $[\alpha]_D^{20}$: +46 ($CHCl_3$;c = 0.5).

Abieta-8,11,13-trien-12-yl-4′-methoxybenzoate (compound **13**): white solid, mp 57–60°C. HRMS: calculated for $C_{28}H_{36}O_3$: 420.2664, found: 420.2662. MS (EI): m/z (rel. int. %): 420 [$M^+$] (7), 285 (3), 147 (2), 135 (100), 107 (2), 77 (3). IR $\nu_{max}$: 2922, 1729, 1607, 1511, 1494, 1273, 1244, 846, 764 $cm^{-1}$. $[\alpha]_D^{20}$: +50 ($CHCl_3$;c = 0.5).

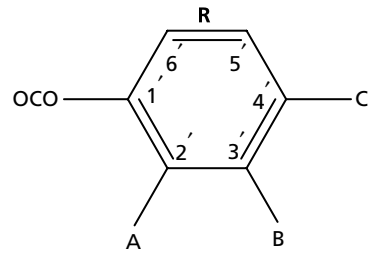
Abieta-8,11,13-trien-12-yl-3′-nitrobenzoate (compound **14**): pale yellow solid, mp 127–130°C. HRMS: calculated for $C_{27}H_{33}NO_4$: 435.2410, found: 435.2406. MS (EI): m/z (rel. int. %): 435 [$M^+$] (71), 420 (84), 405 (5), 378 (12), 364 (9), 350 (29), 338 (61), 324 (36), 310 (12), 285 (37), 150 (100), 147 (11), 120 (28), 104 (25), 69 (38). IR $\nu_{max}$: 2919,

| Compound | R |
|---|---|
| **1** | H |
| **2** | OH |
| **3** | $OCH_3$ |
| **4** | $OSO_2CF_3$ |
| **5** | OCOH |
| **6** | $OCOCH_3$ |
| **7** | $OCOCH_2CH_3$ |
| **8** | $OCOCHClCH_3$ |
| **9** | $OCOCH{=}CH_2$ |

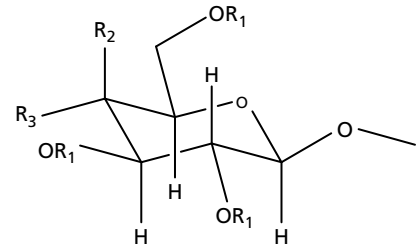


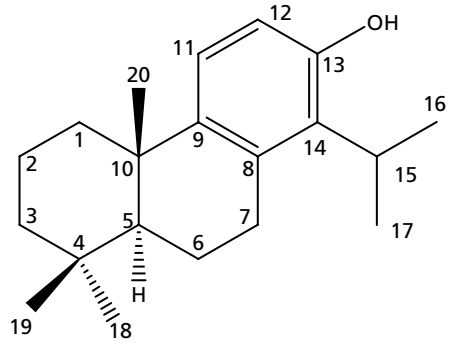

Compounds **1–9**

| Compound | A | B | C |
|---|---|---|---|
| **10** | H | H | H |
| **11** | $CH_3$ | H | H |
| **12** | H | H | $CH_3$ |
| **13** | H | H | $OCH_3$ |
| **14** | H | $NO_2$ | H |
| **15** | H | H | $NO_2$ |




| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| **16** Glucose | H | H | OH |
| **17** | Ac | H | OAc |
| **18** Galactose | H | OH | H |
| **19** | Ac | OAc | H |




Compound **20** (totarol)

**Figure 1** Structure of ferruginol (**2**), its derivatives **1** and **3**–**19**, and totarol (**20**).

**Table 1** $^1$H NMR data of the ferruginol derivatives **4**, **5**, **7** and **8** (400 MHz, $CDCl_3$, $\delta$ in ppm, J in Hz, integrals in parentheses)

| | **4** | **5** | **7** | **8** |
|---|---|---|---|---|
| 1$\beta$ | 2.21 ddd (12.7; 4.0; 1.0) (1 H) | 2.21 ddd (12.7; 4.0; 0.9) (1 H) | 2.17 ddd (12.3; 4.3; 0.6) (1 H) | 2.22 ddd (12.7; 4.1; 1.0) (1 H) |
| 5 | 1.35 dd (12.5; 2.5) (1 H) | 1.36 dd (12.5; 2.5) (1 H) | 1.38 dd (12.5; 2.4) (1 H) | 1.38 dd (12.5; 2.4) (1 H) |
| 7 | 2.90 ddd (17.4; 11.0; 8.0) (1 H) | 2.89 ddd (17.6; 11.3; 7.6) (1 H) | 2.88 ddd (17.4; 11.2; 7.3) (1 H) | 2.89 ddd (17.4; 11.0; 7.6) (1 H) |
| | 2.98 ddd (17.4; 6.9; 1.0) (1 H) | 2.95 ddd (17.6; 7.1; 1.2) (1 H) | 2.94 ddd (17.4; 7.3; 1.7) (1 H) | 2.99 ddd (17.4; 7.6; 1.5) (1 H) |
| 11 | 7.05 s (1 H) | 6.90 s (1 H) | 6.87 s (1 H) | 6.89 s (1 H) |
| 14 | 7.09 s (1 H) | 7.01 s (1 H) | 6.98 s (1 H) | 7.01 s (1 H) |
| 15 | 3.23 dq (6.9) (1 H) | 3.03 dq (6.9) (1 H) | 2.94 dq (7.1) (1 H) | 3.00 dq (6.9) (1 H) |
| 16 | 1.26 d (6.9) (3 H) | 1.22 d (6.9) (3 H) | 1.21 d (7.1) (3 H) | 1.22 d (6.9) (3 H) |
| 17 | 1.28 d (6.9) (3 H) | 1.24 d (6.9(3 H)) | 1.23 d (7.1) (3 H) | 1.23 d (6.9) (3 H) |
| 18 | 1.00 s (3 H) | 0.99 s (3 H) | 0.98 s (3 H) | 0.99 s (3 H) |
| 19 | 0.97 s (3 H) | 0.97 s (3 H) | 0.96 s (3 H) | 0.97 s (3 H) |
| 20 | 1.21 s (3 H) | 1.22 s (3 H) | 1.22 s (3 H) | 1.23 s (3 H) |
| R | | | | |
| 2′ | – | – | 2.63 q (7.6) (2 H) | 4.68 q (7.1) (1 H) |
| 3′ | – | – | 1.33 t (7.6) (3 H) | 1.89 d (7.1) (3 H) |
| OCHO | – | 8.36 s (1 H) | – | – |

1740, 1615, 1558, 1533, 1351, 1279, 1238, 1111, 721 $cm^{-1}$. $[\alpha]_D^{20}$: +41 ($CHCl_3$;c = 0.5).

Abieta-8,11,13-trien-12-yl-4′-nitrobenzoate (compound **15**): pale yellow solid, mp 136–139°C. HRMS: calculated for $C_{27}H_{33}NO_4$: 435.2410, found: 435.2408. MS (EI): m/z (rel. int. %): 435 [$M^+$] (52), 420 (91), 405 (5), 378 (16), 364 (11), 350 (30), 338 (76), 324 (41), 285 (42), 271 (8), 229 (2), 150 (94), 147 (14), 120 (100), 104 (21), 69 (40). IR $\nu_{max}$: 2921, 1743, 1607,1525, 1498, 1346, 1264, 1240, 842, 717 $cm^{-1}$. $[\alpha]_D^{20}$: +54 ($CHCl_3$;c = 0.5).

12-($\beta$-D-glucopyranosyloxy)-abieta-8,11,13-triene (compound **16**): white solid, mp 171–173°C. HRMS: calculated for $C_{26}H_{40}O_6$: 448.2825, found: 448.2830. MS (EI): m/z (rel. int. %): 286 (100), 271 (53), 243 (3), 229 (5), 201 (12), 189 (20), 175 (18), 149 (6), 147 (6), 69 (10). IR $\nu_{max}$: 3370, 2925, 1567, 1495, 1069, 893 $cm^{-1}$. $[\alpha]_D^{20}$: +32 ($CH_3OH$;c = 1.0).

12-(2,3,4,6-Tetra-*O*-acetyl-$\beta$-D-glucopyranosyloxy)-abieta-8,11,13-triene (compound **17**): white solid, mp 166–168°C. HRMS: calculated for $C_{34}H_{48}O_{10}$: 616.3226, found: 616.3247. MS (EI): m/z (rel. int. %): 616 [$M^+$] (8), 331 (99), 286 (83), 271 (24), 229 (11), 201 (15), 169 (100), 145 (25), 127 (42), 109 (99), 97 (15), 69 (22). IR $\nu_{max}$: 2922, 1744, 1501, 1249, 1221, 895 $cm^{-1}$. $[\alpha]_D^{20}$: +8 ($CHCl_3$;c = 0.5).

12-($\beta$-D-galactopyranosyloxy)-abieta-8,11,13-triene (compound 18): white solid, mp 180–183×C. HRMS: calculated for C26H40O6: 448.2825, found: 448.2822. MS (EI): m/z (rel. int. %): 448 [M+] (0.6), 286 (100), 271 (37), 229 (4), 215 (4), 189 (11), 175 (12), 149 (5), 91 (2), 69 (6). IR nmax: 3577, 3387, 2925, 1500, 1055 $cm^{-1}$. $[\alpha]_D^{20}$: +16 ($CH_3OH$;c = 1.0).

12-(2,3,4,6-Tetra-*O*-acetyl-$\beta$-D-galactopyranosyloxy)-abieta-8,11,13-triene (compound **19**): white solid, mp 171–174°C. HRMS: calculated for $C_{34}H_{48}O_{10}$: 616.3247, found: 616.3256. MS (EI): m/z (rel. int. %): 616 [$M^+$] (2), 331 (100), 286 (16), 271 (8), 229 (4), 201 (4), 169 (92), 147 (5), 127 (14), 109 (31), 69 (5). IR $\nu_{max}$: 2920, 1744, 1501, 1247, 1229 $cm^{-1}$. $[\alpha]_D^{20}$: +66 ($CHCl_3$;c = 0.5).

Totarol (compound **20**): orange solid, mp 125–127°C .

Ferruginol (**2**) is an abietane diterpene presenting a free OH group at C-12. From the natural product, several aliphatic and aromatic esters as well as glycosides were prepared and assessed orally at 20 mg $kg^{-1}$ in the HCl/ethanol-induced gastric lesion model in mice. The closely related compound totarol (**20**) was included in the study as it differs from ferruginol in the position of the phenolic hydroxyl and isopropyl group in the aromatic moiety of the molecule (see Figure 1). In addition, the cytotoxicity of the compounds was evaluated in AGS cells and human fibroblasts.

Several biological activities have been reported for ferruginol, including antimicrobial effects (Politi et al 2003), inhibitory effects on Epstein-Barr virus early antigen activation induced by 12-*O*-tetradecanoylphorbol 13-acetate (Iwamoto et al 2003), cardioactive activity (Kolak et al 2001; Ulubelen et al 2002) and hypotensive effects (Ulubelen et al 2000).

Totarol was reported as an antioxidant (Haraguchi et al 1996) and antimicrobial plant product (Solís et al 2004). The antibacterial effect of totarol and its derivatives was studied by Evans et al (2000) and it was shown that totarol uncoupled oxidative phosphorylation in isolated mitochondria.

The compounds **1**, **2**, **3**, **6** and **20** have been previously reported from natural sources (Ying & Kubo 1991). Ferruginol was previously reported as a constituent of the resin of *Podocarpus ferrugineus*, *Podocarpus totara*, *Dacrydium* spp., *Cupressus* spp., *Cryptomeria japonica*, *Salvia syriaca* and the roots of *Inula royleana* (Dictionary of Natural Products on CD ROM 2005), *Cephalotaxus harringtonia* (Politi et al 2003), *Thuja standishii* (Iwamoto et al 2003), *Salvia eriophora* (Ulubelen et al 2002), *Salvia amplexicaulis* (Kolak et al 2001) and *Salvia syriaca* (Ulubelen et al 2000). Compound **9** was previously described in a patent for photosensitive material (Shida et al 1996), while the benzoate **10** was prepared by King et al (1957) to compare synthetic and natural ferruginol as crystalline benzoates. Compounds **4**, **5**, **7**, **8** and **11**–**19** are described here for the first time.

**Table 2** $^{1}$H NMR data of ferruginol derivatives **10–15** (400 MHz, $CDCl_3$, $\delta$ in ppm, J in Hz, integrals in parentheses)

| | **10** | **11** | **12** | **13** | **14** | **15** |
|---|---|---|---|---|---|---|
| 1$\beta$ | 2.18 dt (12.5; 4.0) (1 H) | 2.23 dt (12.7; 4.0) (1 H) | 2.25 ddd (12.5; 4.2; 1.2) (1 H) | 2.18 ddd (12.2; 4.5; 0.9) (1 H) | 2.16 dt (12.2; 4.1) (1 H) | 2.17 dt (12.2; 4.0) (1 H) |
| 5 | 1.37 dd (12.4; 2.5) (1 H) | 1.42 dd (12.5; 2.2) (1 H) | 1.39 dd (12.5; 2.5) (1 H) | 1.39 dd (12.5; 2.5) (1 H) | 1.37 dd (12.5; 2.5) (1 H) | 1.36 dd (12.5; 2.5) (1 H) |
| 7 | 2.91 ddd (17.4; 11.0; 7.6) (1 H); 2.98 ddd (17.4; 7.6; 1.2) (1 H) | 2.92 ddd (17.4; 10.8; 8.1) (1 H); 2.97 ddd (17.4; 8.1; 1.5) (1 H) | 2.92 ddd (17.4; 11.0; 7.3) (1 H); 2.98 ddd (17.4; 7.3; 1.5) (1 H) | 2.91 ddd (17.4; 11.3; 7.6) (1 H); 2.98 ddd (17.4; 7.6; 1.2) (1 H) | 2.91 ddd (17.6; 11.3; 7.8) (1 H); 2.96 ddd (17.6; 7.8; 1.5) (1 H) | 2.88 ddd (17.6; 11.3; 7.6) (1 H); 2.93 ddd (17.6; 7.6; 1.7) (1 H) |
| 11 | 6.97 s (1 H) | 6.99 s (1 H) | 7.01 s (1 H) | 7.01 s (1 H) | 6.99 s (1 H) | 6.97 s (1 H) |
| 14 | 6.99 s (1 H) | 7.04 s (1 H) | 7.03 s (1 H) | 7.03 s (1 H) | 7.04 s (1 H) | 7.01 s (1 H) |
| 15 | 3.05 dq (7.1) (1 H) | 3.03 dq (7.1) (1 H) | 3.01 dq (7.1) (1 H) | 3.03 dq (7.1) (1 H) | 2.99 dq (6.9) (1 H) | 2.95 dq (6.9) (1 H) |
| 16 | 1.19 d (7.1) (3 H) | 1.24 d (7.1) (3 H) | 1.23 d (7.1) (3 H) | 1.24 d (7.1) (3 H) | 1.22 d (6.9) (3 H) | 1.20 d (6.9) (3 H) |
| 17 | 1.21 d (7.1) (3 H) | 1.26 d (7.1) (3 H) | 1.25 d (7.1) (3 H) | 1.26 d (7.1) (3 H) | 1.24 d (6.9) (3 H) | 1.21 d (6.9) (3 H) |
| 18 | 0.95 s (3 H) | 1.00 s (3 H) | 1.00 s (3 H) | 1.00 s (3 H) | 0.97 s (3 H) | 0.96 s (3 H) |
| 19 | 0.93 s (3 H) | 0.98 s (3 H) | 0.97 s (3 H) | 0.98 s (3 H) | 0.95 s (3 H) | 0.93 s (3 H) |
| 20 | 1.20 s (3 H) | 1.26 s (3 H) | 1.25 s (3 H) | 1.26 s (3 H) | 1.22 s (3 H) | 1.21 s (3 H) |
| R | | | | | | |
| 2′ | 8.22 dd (7.8; 1.2) (1 H) | – | 8.15 dbr (8.1) (1 H) | 8.22 d (8.6) (1 H) | 9.06 dd (1.9; 1.8) (1 H) | 8.39 ddbr (9.0; 2.4) (1 H) |
| 3′ | 7.51 dd (7.8; 7.3) (1 H) | 7.36 brd (7.6) (1 H) | 7.36 dbr (8.3) (1 H) | 7.04 d (8.6) (1 H) | – | 8.35 ddbr (9.0; 2.4) (1 H) |
| 4′ | 7.63 ddbr (7.3; 1.2) (1 H) | 7.52 dd (7.6; 7.3) (1 H) | – | – | 8.49 ddd (8.1; 2.2; 1.2) (1 H) | – |
| 5′ | 7.51 dd (7.8; 7.3) (1 H) | 7.37 dd (7.8; 7.3) (1 H) | 7.36 dbr (8.3) (1 H) | 7.04 d (8.6) (1 H) | 7.74 dd (8.0; 7.9) (1 H) | 8.35 ddbr (9.0; 2.4) (1 H) |
| 6′ | 8.22 dd (7.8; 1.2) (1 H) | 8.22 d (7.8) (1 H) | 8.15 dbr (8.1) (1 H) | 8.22 d (8.6) (1 H) | 8.55 ddd (7.9; 1.3;1.3) (1 H) | 8.39 ddbr (9.0; 2.4) (1 H) |
| $CH_3$ | – | 2.72 s (3 H) | 2.50 s (3 H) | – | – | – |
| $OCH_3$ | – | – | – | 3.94 s (3 H) | – | – |

**Table 3** $^{1}$H NMR data of the ferruginol derivatives **16**–**19** (400 MHz, **16** and **18** in MeOH-$d_4$; **17** and **19** in $CDCl_3$, δ in ppm, J in Hz, integrals in parentheses)

| H | 16 | 17 | 18 | 19 |
|---|---|---|---|---|
| 1$\beta$ | 2.32 ddd (12.7; 4.0; 1.0) (1 H) | 2.21 ddd (12.5; 4.1; 1.2) (1 H) | 2.33 ddd (12.5; 4.3; 0.9) (1 H) | 2.21 ddd (12.5; 3.7; 1.0) (1 H) |
| 5 | 1.37 dd (13.2; 3.4) (1 H) | 1.36 dd (12.5; 2.2) (1 H) | 1.40 dd (12.9; 3.4) (1 H) | 1.36 dd (12.5; 2.2) (1 H) |
| 7 | 2.76 ddd (17.1; 11.5; 7.6) (1 H); 2.86 ddd (17.1; 5.6; 1.0) (1 H) | 2.85 ddd (17.1; 11.3; 7.3) (1 H); 2.88 ddd (17.1; 6.9; 1.5) (1 H) | 2.78 ddd (17.1; 11.7; 7.6) (1 H); 2.85 ddd (17.1; 6.6; 1.0) (1 H) | 2.85 ddd (17.1; 11.3; 7.3) (1 H); 2.89 ddd (17.1; 6.9; 1.5) (1 H) |
| 11 | 7.05 s (1 H) | 6.94 s (1 H) | 7.06 s (1 H) | 6.99 s (1 H) |
| 14 | 6.84 s (1 H) | 6.90 s (1 H) | 6.84 s (1 H) | 6.90 s (1 H) |
| 15 | 3.40 dq (6.9; 6.9) (1 H) | 3.20 dq (6.9; 6.9) (1 H) | 3.46 dq (6.9; 6.9) (1 H) | 3.22 dq (6.9; 6.9) (1 H) |
| 16 | 1.17 d (6.9) (3 H) | 1.17 d (6.9) (3 H) | 1.17 d (6.9) (3 H) | 1.17 d (6.9) (3 H) |
| 17 | 1.20 d (6.9) (3 H) | 1.19 d (6.9) (3 H) | 1.19 d (6.9) (3 H) | 1.19 d (6.9) (3 H) |
| 18 | 0.97 s (3 H) | 0.98 s (3 H) | 0.98 s (3 H) | 0.98 s (3 H) |
| 19 | 0.96 s (3 H) | 0.96 s (3 H) | 0.96 s (3 H) | 0.96 s (3 H) |
| 20 | 1.19 s (3 H) | 1.22 s (3 H) | 1.19 s (3 H) | 1.23 s (3 H) |
| Sugar | | | | |
| 1′ | 4.80 d (7.6) (1 H) | 5.02 d (7.8) (1 H) | 4.76 d (7.8) (1 H) | 4.97 d (8.1) (1 H) |
| 2′ | | 5.33–5.35 m* | 3.82 dd (9.8; 7.8) (1 H) | 5.56 dd (10.5; 7.8) (1 H) |
| 3′ | 3.40–3.50 m (4 H) | 5.20 dd (9.8; 9.5) (1 H) | 3.59 dd (9.8; 3.4) (1 H) | 5.14 dd (10.5; 3.4 ) (1 H) |
| 4′ | | 5.33–5.35 m* | 3.93 dd (3.2; 0.5) (1 H) | 5.49 dd (3.4; 1.0) (1 H) |
| 5′ | | 3.88 ddd (7.6; 5.4; 2.5) (1 H) | 3.64 ddd (6.5; 6.5; 0.7) (1 H) | 4.08 ddd (6.8; 5.9; 0.7) (1 H) |
| 6′ | 3.90 dd (12.0; 5.1) (1 H) | 4.28 dd (12.5; 5.4) (1 H) | 3.77 d (6.4) (1 H) | 4.27 dd (11.5; 5.9) (1 H) |
| | 3.73 dd (12.0; 1.7) (1 H) | 4.22 dd (12.5; 2.5) (1 H) | * | 4.19 dd (11.5; 6.8) (1 H) |
| OAc | – | 2.12 s (3 H); 2.10 s (3 H); 2.09 s (3 H); 2.08 s (3 H) | – | 2.24 s (3 H); 2.11 s (3 H); 2.09 s (3 H); 2.05 s (3 H) |

*Signals overlapping.

**Table 4** $^{13}$C NMR data of the ferruginol derivatives **4**, **5**, **7** and **8** (100 MHz, $CDCl_3$, $\delta$ in ppm, J in Hz)

| | 4 | 5 | 7 | 8 |
|---|---|---|---|---|
| 1 | 38.65 t | 38.79 t | 38.81 t | 38.83 t |
| 2 | 18.83 t | 19.00 t | 19.09 t | 19.06 t |
| 3 | 41.65 t | 41.63 t | 41.70 t | 41.68 t |
| 4 | 33.44 s | 33.44 s | 33.45 s | 33.47 s |
| 5 | 49.92 d | 50.07 d | 50.11 d | 50.09 d |
| 6 | 19.15 t | 19.20 t | 19.25 t | 19.23 t |
| 7 | 29.90 t | 29.94 t | 30.01 t | 30.01 t |
| 8 | 135.82 s | 133.75 s | 132.92 s | 133.59 s |
| 9 | 149.91 s | 149.16 s | 148.77 s | 149.08 s |
| 10 | 37.76 s | 37.65 s | 37.62 s | 37.67 s |
| 11 | 116.97 d | 117.36 d | 117.98 d | 117.43 d |
| 12 | 145.46 s | 145.52 s | 146.23 s | 145.87 s |
| 13 | 137.41 s | 136.61 s | 136.60 s | 136.55 s |
| 14 | 127.87 d | 127.21 d | 126.82 d | 126.98 d |
| 15 | 26.75 d | 27.00 d | 27.15 d | 26.91 d |
| 16 | 23.15 q | 22.96 q | 22.95 q | 23.06 q |
| 17 | 23.19 q | 23.10 q | 23.08 q | 23.09 q |
| 18 | 33.21 q | 33.26 q | 33.31 q | 33.31 q |
| 19 | 21.58 q | 21.00 q | 21.62 q | 21.63 q |
| 20 | 24.72 q | 24.79 q | 24.80 q | 24.80 q |
| R | | | | |
| 1′ | 120.01 s | – | 173.26 s | 168.93 s |
| 2′ | – | – | 27.80 t | 52.52 d |
| 3′ | – | – | 9.30 q | 21.53 q |
| OCHO | – | 160.18 d | – | – |

Totarol is a constituent of *Podocarpus* spp., *Dacrydium cupressinum*, *Tetraclinis articulata* and *Thujopsis dolabrata*, while dehydroabietane (**1**) was isolated from *Pinus pallasiana* and *Podocarpus ferrugineus* (Dictionary of Natural Products on CD ROM 2005).

The greatest gastroprotective effect was provided by compounds **1**, **8**, **9**, **17** and **18**, being as active as lansoprazole at 20 mg $kg^{-1}$ and reducing gastric lesions by 69, 76, 67, 72 and 61%, respectively (Table 7). At the given dose, the gastroprotective effect of compounds **7**, **15** and **16** did not differ statistically from the untreated controls.

Compound **1** was prepared to assess the contribution of the phenolic OH in the gastroprotective effect of ferruginol. As the percentage lesion reduction for both compounds was not statistically different, we can conclude that the OH group is not a structural requirement for the gastroprotective effect. The hydrocarbon **1** was less cytotoxic than ferruginol, both compounds being more active against AGS cells than against fibroblasts. Methoxylation of the OH group afforded the methyl ether (compound **3**), which had a similar gastroprotective effect as the precursor **2** but with lower cytotoxicity. The position of the OH and isopropyl group in the aromatic moiety of the compound did not elicit significant differences in the gastroprotective effect or in cytotoxicity, as can be seen by comparing the effects of ferruginol (compound **2**) and totarol (compound **20**).

In the aliphatic esters (compounds **6**–**9**), a significant increase in the gastroprotective effect compared with the control was observed for the C3 chain compounds bearing a chlorine atom or double bond (compounds **8** and **9**). The effect, however, was similar to that of the parent compound ferruginol

**Table 5** $^{13}C$ NMR data of the ferruginol derivatives **10–15** (100 MHz, $CDCl_3$, $\delta$ in ppm, J in Hz)

| | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|
| 1 | 38.37 t | 38.86 t | 38.84 t | 38.86 t | 38.87 t | 38.87 t |
| 2 | 19.80 t | 19.11 t | 19.12 t | 19.14 t | 19.09 t | 19.06 t |
| 3 | 41.86 t | 41.76 t | 41.72 t | 41.74 t | 41.71 t | 41.68 t |
| 4 | 33.54 s | 33.50 s | 33.47 s | 33.48 s | 33.47 s | 33.48 s |
| 5 | 50.17 d | 50.16 d | 50.15 d | 50.16 d | 50.12 d | 50.11 d |
| 6 | 19.90 t | 19.17 t | 19.25 t | 19.27 t | 19.25 t | 19.22 t |
| 7 | 30.02 t | 30.06 t | 30.04 t | 30.06 t | 30.04 t | 30.01 t |
| 8 | 133.30 s | 133.07 s | 132.98 s | 132.92 s | 133.71 s | 133.72 s |
| 9 | 149.03 s | 148.92 s | 148.85 s | 148.83 s | 149.17 s | 149.18 s |
| 10 | 37.60 s | 37.70 s | 37.67 s | 37.68 s | 37.62 s | 37.71 s |
| 11 | 118.00 d | 118.15 d | 118.17 d | 118.25 d | 117.81 d | 117.77 d |
| 12 | 146.47 s | 146.42 s | 146.47 s | 146.51 s | 146.09 s | 146.06 s |
| 13 | 136.80 s | 136.92 s | 136.83 s | 136.88 s | 136.60 s | 136.50 s |
| 14 | 127.50 d | 126.95 d | 126.90 d | 126.90 d | 127.17 d | 127.16 d |
| 15 | 27.45 d | 27.26 d | 27.33 d | 27.35 d | 27.38 d | 27.38 d |
| 16 | 23.14 q | 23.12 q | 22.98 q | 23.01 q | 23.05 q | 22.98 q |
| 17 | 23.14 q | 23.25 q | 23.12 q | 23.14 q | 23.16 q | 23.10 q |
| 18 | 33.54 q | 33.37 q | 33.31 q | 33.33 q | 33.30 q | 33.27 q |
| 19 | 21.78 q | 21.68 q | 21.63 q | 21.65 q | 21.64 q | 21.64 q |
| 20 | 24.90 q | 24.90 q | 24.82 q | 24.84 q | 24.83 q | 24.81 q |
| RCOOR | | | | | | |
| | 165.02 s | 166.24 s | 165.55 s | 165.22 s | 163.53 s | 163.70 s |
| 1′ | 130.00 s | 129.06 s | 127.20 s | 122.30 s | 131.74 s | 135.31 s |
| 2′ | 130.05 d | 141.26 s | 130.20 d | 132.24 d | 125.08 d | 131.24 d |
| 3′ | 128.50 d | 131.98 d | 129.30 d | 113.88 d | 148.55 s | 123.75 d |
| 4′ | 133.30 d | 132.53 d | 144.16 s | 163.81 s | 127.83 d | 150.91 s |
| 5′ | 128.50 d | 125.96 d | 129.30 d | 113.88 d | 129.94 d | 123.75 d |
| 6′ | 130.05 d | 131.10 d | 130.20 d | 132.24 d | 135.70 d | 131.24 d |
| $OCH_3$ | – | – | – | 55.52 q | – | – |
| $CH_3$ | – | 22.00 q | 21.75 q | – | – | – |

**Table 6** $^{13}C$ NMR data of the ferruginol derivatives **16–19** (100 MHz, **16** and **18** in MeOH-$d_4$; **17** and **19** in $CDCl_3$, $\delta$ in ppm, J in Hz)

| | 16 | 17 | 18 | 19 |
|---|---|---|---|---|
| 1 | 38.67 t | 38.76 t | 38.68 t | 38.76 t |
| 2 | 19.01 t | 19.13 t | 19.01 t | 19.15 t |
| 3 | 41.57 t | 41.63 t | 41.56 t | 41.64 t |
| 4 | 33.01 s | 33.45 s | 32.99 s | 33.44 s |
| 5 | 50.76 d | 50.37 d | 50.76 d | 50.37 d |
| 6 | 19.01 t | 19.37 t | 19.01 t | 19.39 t |
| 7 | 29.62 t | 29.89 t | 29.60 t | 29.90 t |
| 8 | 128.72 s | 130.29 s | 128.67 s | 130.30 s |
| 9 | 148.12 s | 148.52 s | 148.07 s | 148.51 s |
| 10 | 37.55 s | 37.75 s | 37.54 s | 37.75 s |
| 11 | 111.41 d | 111.86 d | 112.51 d | 112.19 d |
| 12 | 152.95 s | 150.41 s | 153.04 s | 152.49 s |
| 13 | 135.11 s | 135.46 s | 135.21 s | 135.50 s |
| 14 | 125.80 d | 126.57 d | 125.74 d | 126.47 d |
| 15 | 25.70 d | 26.00 d | 25.63 d | 25.87 d |
| 16 | 22.06 q | 22.90 q | 22.08 q | 22.95 q |
| 17 | 22.27 q | 23.09 q | 22.26 q | 23.18 q |
| 18 | 32.47 q | 33.26 q | 32.44 q | 33.28 q |
| 19 | 20.74 q | 21.62 q | 20.69 q | 21.62 q |
| 20 | 23.96 q | 25.03 q | 23.92 q | 25.04 q |
| Sugar | | | | |
| 1′ | 101.99 d | 100.00 d | 102.70 d | 100.76 d |
| 2′ | 73.73 d | 71.25 d | 71.16 d | 68.84 d |
| 3′ | 76.67 d | 72.96 d | 73.85 d | 71.04 d |
| 4′ | 70.16 d | 68.46 d | 68.80 d | 67.31 d |
| 5′ | 77.02 d | 72.06 d | 75.38 d | 71.10 d |
| 6′ | 61.28 t | 62.42 t | 60.86 t | 62.03 t |
| OAc | | | | |
| $OCCH_3$ | – | 169.17 s; 169.83 s; 170.27 s; 170.66 s | – | 169.23 s; 170.12 s; 170.28 s; 170.41 s |
| $OCCH_3$ | – | 20.59 q; 20.61 q; 20.63 q; 20.68 q | – | 20.75 q; 20.68 q; 20.65 q; 20.57 q |

(**2**). In the aromatic esters **10–15**, remarkable differences were found in the gastroprotective effect, the most active being the 2-methyl-, 4-methyl- and the 3-nitrobenzoate (percentage lesion reduction of 54, 47 and 59%, respectively), with effects similar to that of ferruginol but with much lower cytotoxicity.

Glycosidation yielded the active compounds **17**, **18** and **19**, which reduced gastric lesions by 72, 61 and 52%, respectively, at 20 mg $kg^{-1}$. Of particular interest was the tetraacetate **17**, which was more active than ferruginol but displayed much lower cytotoxicity. No relation was observed between lipophilicity values and the gastroprotective effect.

The greatest cytotoxicity towards AGS cells was observed for compounds **2**, **5–9**, **16**, **18** and **20** (IC50 over the range 18–44 $\mu$M), while compounds **10–15** (which had the highest lipophilicity values) and **17** were the least cytotoxic (IC50 values >1000 $\mu$M). Compounds **2**, **5–9**, **16**, **18** and **20** were the most toxic against fibroblasts (IC50 over the range 19–56 $\mu$M), with a correlation with the AGS cells. Compounds **9–15**, **17** and **19** showed low cytotoxicity (IC50 values >1000 $\mu$M), the derivative **19** being almost 5-times more active against AGS cells than towards fibroblasts.

Diterpenes belonging to the abietane skeleton have been shown to display gastroprotective effects in animal models. A dehydroabietic acid derivative, ecabet sodium, is being developed as a therapeutic agent in Japan (Onoda et al 1990). The mechanism of action of this compound has been related both to an increase in gastric mucus (Onoda et al 1990) as well as to a dose-dependent increase in prostaglandin $E_2$ production (Ichikawa et al 2000). According to Ito et al (1991), ecabet exerts its effects mainly by local action but not by systemic action. Recently, Rodríguez et al (2006) reported that the mechanism underlying the gastroprotective activity of ferruginol in-vitro involved an increase in the prostaglandin $E_2$ content and the inhibition of lipid peroxidation. In addition, we have observed that ferruginol increases the glutathione content and displays an antisecretory effect in mice (data not shown).

A recent study on the gastroprotective effect and cytotoxicity of abietanes was undertaken with semisynthetic C-18 dehydroabietic acid derivatives (Sepúlveda et al 2005). The comparison of the compounds was performed at a single oral dose of 100 mg $kg^{-1}$, some 5-times greater than the present study and included esters and aromatic amides. The greatest gastroprotective effect was provided by dehydroabietanol, its corresponding aldehyde, dehydroabietic acid and its methyl ester, *N*-(*m*-nitrophenyl)-, *N*-(*o*-chlorophenyl)- and *N*-(*p*-iodophenyl) abieta-8,11,13-trien-18-amide, *N*-2-aminothiazolyl- and *N*-benzylabieta-8,11, 13-trien-18-amide, being as active as lansoprazole at 20 mg $kg^{-1}$ and

**Table 7** Gastroprotective effect of ferruginol (**2**), the semisynthetic derivatives **1**, **3**, **6–19**, totarol (**20**) and lansoprazole at 20 mg kg$^{-1}$ on HCl/ethanol-induced gastric lesions in mice and cytotoxicity towards AGS cells and human fibroblasts

| Compound | Lipophilicity (log P) | n | Lesion index (mm) | % Lesion reduction | Cytotoxicity IC50 ($\mu$M) | |
|---|---|---|---|---|---|---|
| | | | | | AGS | Fibroblasts |
| **1** | 7.11 | 7 | 10.5 ± 6.7* | 69 | 491 ± 27 | 217 ± 14 |
| **2** | 6.46 | 8 | 15.5 ± 7.7* | 55 | 27 ± 2 | 23 ± 2 |
| **3** | 6.72 | 7 | 17.1 ± 5.2* | 50 | 275 ± 15 | 159 ± 9 |
| **5** | 6.32 | – | – | – | 21 ± 1 | 46 ± 2 |
| **6** | 6.43 | 7 | 21.9 ± 6.0* | 36 | 44 ± 3 | 38 ± 2 |
| **7** | 7.08 | 7 | 31.9 ± 7.9 | 7 | 21 ± 2 | 22 ± 3 |
| **8** | 7.45 | 8 | 8.4 ± 4.3* | 76 | 30 ± 2 | 24 ± 1 |
| **9** | 7.11 | 8 | 11.3 ± 8.2* | 67 | 27 ± 2 | 56 ± 3 |
| **10** | 8.33 | 7 | 21.3 ± 7.9* | 38 | >1000 | >1000 |
| **11** | 8.82 | 8 | 15.8 ± 7.6* | 54 | >1000 | >1000 |
| **12** | 8.82 | 7 | 18.1 ± 6.7* | 47 | >1000 | >1000 |
| **13** | 8.20 | 7 | 20.7 ± 4.3* | 40 | >1000 | >1000 |
| **14** | 8.09 | 7 | 14.1 ± 6.6* | 59 | >1000 | >1000 |
| **15** | 8.09 | 7 | 23.6 ± 5.6* | 31 | >1000 | >1000 |
| **16** | 4.16 | 7 | 23.1 ± 9.4* | 33 | 31 ± 2 | 19 ± 1 |
| **17** | 5.53 | 7 | 9.6 ± 4.9* | 72 | >1000 | >1000 |
| **18** | 4.16 | 7 | 13.4 ± 7.4* | 61 | 23 ± 2 | 40 ± 3 |
| **19** | 5.53 | 7 | 16.4 ± 5.8* | 52 | 225 ± 13 | >1000 |
| **20** | 6.46 | 7 | 14.1 ± 6.2* | 59 | 18 ± 2 | 19 ± 1 |
| Lansoprazole | – | 8 | 9.8 ± 3.1* | 71 | 162 ± 10 | 306 ± 17 |
| Control | – | 8 | 34.4 ± 7.2* | – | – | – |

Results are expressed as mean ± s.d. *$P < 0.01$ significantly different compared with the control (analysis of variance followed by Dunnett's test).

reducing the lesion index by at least 75%. In the compound series including the alcohol, ester, aldehyde, acid and methyl ester at C-18, the greatest activity was related to the presence of an alcohol, aldehyde, acid or methyl ester at C-18, the activity being strongly reduced after esterification (Sepúlveda et al 2005).

When compared with other diterpenes and their semisynthetic derivatives such as solidagenone (Schmeda-Hirschmann et al 2002; Rodríguez et al 2005), labdanes from *Araucaria imbricata* (Schmeda-Hirschmann et al 2005) and other naturally occurring terpenoids (Schmeda-Hirschmann & Yesilada 2005), the gastroprotective effect of several ferruginol derivatives reported in this study was greater or comparable with that of lansoprazole at the same oral dose. The best activity/cytotoxicity ratio was found for the derivative **17**, with a lesion index comparable with lansoprazole at 20 mg kg$^{-1}$ and cytotoxicity >1000 $\mu$M towards MRC-5 and AGS cells.

## Conclusions

This work reports the gastroprotective activity of 18 semisynthetic ferruginol derivatives and totarol. Some derivatives displayed a gastroprotective effect that was comparable with the reference drug lansoprazole at the same dose but showed lower cytotoxicity than the parent compound ferruginol. These promising results encourage further pharmacological studies of these compounds as potential gastroprotective drugs. Further work is necessary to determine the mechanism of action of the active low cytotoxic ferruginol derivatives.

## References


Ann, M., Correa, P., Aparecida, M., Possenti, A., Ferreira, R. A., Ferreira, E., Garcia, V. L., de Carvalho, J. E. (2002) Antiulcerogenic activity of some sesquiterpene lactones isolated from *Artemisia annua*. *Planta Med.* **68**: 515–518

Arrieta, J., Benitez, J., Flores, E., Castillo, C., Navarrete, A. (2003) Purification of gastroprotective triterpenoids from the stem bark of *Amphipterygium adstringens*; role of prostaglandins, sulfhydryls, nitric oxide and capsaicin-sensitive neurons. *Planta Med.* **69**: 905–909

*Dictionary of natural products on CD-ROM* (2005) Version 13:2 2005. Chapman and Hall/CRC, Boca Raton, FL

Evans, G. B., Furneaux, R. H., Gainsford, G. J., Murphy, M. P. (2000) The synthesis and antibacterial activity of totarol derivatives. Part 3: modification of ring-B. *Bioorg. Med. Chem.* **8**: 1663–1675

Favier, L. S., Maria, A. O. M., Wendel, G. H., Borkowski, E. J., Giordano, O. S., Pelzer, L., Tonn, C. E. (2005) Anti-ulcerogenic activity of xanthanolide sesquiterpenes from *Xanthium cavanillesii* in rat. *J. Ethnopharmacol.* **100**: 260–267

Haraguchi, H., Ishikawa, H., Sakai, S., Ying, B. P., Kubo, I. (1996) Inhibition of lipid peroxidation by diterpenoid from *Podocarpus nagi*. *Experientia* **52**: 573–576

Hiruma-Lima, C. A., Gracioso, J. S., Toma, W., Almeida, A. B., Paula, A. C., Brasil, D. S. B., Muller, A. H., Souza-Brito, A. R. M. (2001) Gastroprotective effects of aparisthman, a diterpene isolated

from *Aparisthmium cordatum*, on experimental gastric ulcer model in rat and mice. *Phytomedicine* **8**: 94–100

Ichikawa, T., Ishihara, K., Hayashida, H., Hiruma, H., Saigenji, K., Hotta, K. (2000) Effects of ecabet sodium, a novel gastroprotective agent, on mucin metabolism in rat gastric mucosa. *Dig. Dis. Sci*. **45**: 606–613

Ito, Y., Fukushima, T., Sugawara, Y., Takaiti, O., Nakamura, S. (1991) Metabolic fate of a new anti-ulcer drug (1)-(1*R*,4a*S*,10a*R*)-1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-6-sulfo-1-phenanthrenecarboxylic acid 6-sodium salt pentahydrate (TA-2711). I. Disposition, metabolism and protein binding in rats and dogs. *J. Pharmacobiodyn*. **14**: 533–546

Iwamoto, M., Minami, T., Tokuda, H., Ohtsu, H., Tanaka, R. (2003) Potential antitumor promoting diterpenoids from the stem bark of *Thuja standishii*. *Planta Med*. **69**: 69–72

King, F. E., King, T. J., Topliss, J. G. (1957) Total synthesis of (+)-ferruginol. *J. Chem. Soc*. 573–577

Kolak, U., Ari, S., Birman, H., Hasancebi, S., Ulubelen, A. (2001) Cardioactive diterpenoids from the roots of *Salvia amplexicaulis*. *Planta Med*. **67**: 761–763

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

Onoda, Y., Takido, M., Magaribuchi, T., Tamaki, H. (1990) Effects of 12-sulfodehydroabietic acid monosodium salt (TA-2711), a new anti-ulcer agent, on gastric mucosal lesions induced by necrotizing agents and gastric mucosal defensive factors in rats. *Jpn. J. Pharmacol*. **52**: 631–638

Politi, M., Braca, A., De Tommasi, N., Morelli, I., Manunta, A., Battinelli, L., Mazzanti, G. (2003) Antimicrobial diterpenes from the seeds of *Cephalotaxus harringtonia* var. *drupacea*. *Planta Med*. **69**: 468–470

Reyes, M., Schmeda-Hirschmann, G., Razmilic, I., Theoduloz, C., Yáñez, T., Rodríguez, J. A. (2005) Gastroprotective activity of sesquiterpene derivatives from *Fabiana imbricata*. *Phytother. Res*. **19**: 1038–1042

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

Rodríguez, J. A., Theoduloz, C., Yáñez, T., Becerra, J., Schmeda-Hirschmann, G. (2006) Gastroprotective and ulcer healing effects of ferruginol in mice and rat: assessment of its mechanism of action using in vitro model. *Life Sci*. **78**: 2503–2509

Schmeda-Hirschmann, G., Yesilada, E. (2005) Traditional medicine and gastroprotective crude drugs. *J. Ethnopharmacol*. **100**: 61–66

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

Schmeda-Hirschmann, G., Astudillo, L., Sepúlveda, B., Rodríguez, J. A., Theoduloz, C., Yáñez, T., Palenzuela, J. A. (2005) Gastroprotective effect and cytotoxicity of natural and semisynthetic labdane diterpenes from *Araucaria araucana*. *Z. Naturforsch.* C **60**: 511–522

Sepúlveda, B., Astudillo, L., Rodríguez, J., Yáñez, T., Theoduloz, C., Schmeda-Hirschmann, G. (2005) Gastroprotective and cytotoxic effect of dehydroabietic acid derivatives. *Pharmacol. Res*. **52**: 429–437

Shida, N., Ushirogouchi, T., Naito, T., Nakase, M. (1996) (Kabushiki Kaisha Toshiba, Japan). Photosensitive material. Ger. Offen., 371 pp. CODEN: GWXXBX DE 19525221 A1 19960125 Patent written in German. Application: DE 95–19525221 19950711. Priority: JP 94–158512 19940711. CAN 124:302576 AN 1996:190901

Silva Melo, P., Duran, N., Hiruma-Lima, C. A., Souza-Brito, A. R. M., 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

Solís, C., Becerra, J., Flores, C., Robledo, J., Silva, M. (2004) Antibacterial and antifungal terpenes from *Pilgerodendron uviferum* (D. Don) Florin. *J. Chil. Chem. Soc*. **49**: 157–161

Ulubelen, A., Oksuz, S., Kolak, U., Birman, H., Voelter, W. (2000) Cardioactive terpenoids and a new rearranged diterpene from *Salvia syriaca*. *Planta Med*. **66**: 627–629

Ulubelen, A., Birman, H., Oksuz, S., Topcu, G., Kolak, U., Barla, A., Voelter, W. (2002) Cardioactive diterpenes from the roots of *Salvia eriophora*. *Planta Med*. **68**: 818–821

Ying, B., Kubo, I. (1991) Complete $^{1}H$ and $^{13}C$ NMC assignments of totarol and its derivatives. *Phytochemistry* **6**: 1951–195