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Gastroprotective activity of *ent*-beyerene derivatives in mice: Effects on gastric secretion, endogenous prostaglandins and non-protein sulfhydryls

Teresa Parra [a], Julio Benites [b], Lina M. Ruiz [c], Beatriz Sepulveda [d], Mario Simirgiotis [e], Carlos Areche [a,*]

[a] Departamento de Química, Facultad de Ciencias, Universidad de Chile, Santiago, Chile
[b] Facultad de Ciencias de la Salud, Universidad Arturo Prat., Iquique, Chile
[c] Facultad de Ciencias de la Salud, Universidad Autónoma de Chile, Santiago, Chile
[d] Departamento de Ciencias Químicas, Facultad de Ciencias Exactas, Universidad Andres Bello, Quillota 980, Viña del Mar, Chile
[e] Laboratorio de Productos Naturales, Departamento de Química, Facultad de Ciencias Básicas, Universidad de Antofagasta, Antofagasta, Chile

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ABSTRACT

Seventeen compounds (**2**–**18**) synthetized from the diterpenoid *ent*-beyer-15-en-18-ol (**1**) isolated from aerial part of *Baccharis tola* were tested for their gastroprotective activity on the model of HCl/EtOH-induced gastric lesions in mice. Furthermore cytotoxicity test toward fibroblasts and AGS cells were performed. The results showed that compound **1** (ED_{50} = 50 mg/kg), **2**, **6** and **13** were the most active regarding gastroprotective activity. Compounds **8**–**10** and **17**–**18** showed the lowest cytotoxicity toward fibroblasts and AGS cells. Regarding to mode of gastroprotective action, the effect elicited by **6** (50 mg/kg) was reversed by Indomethacin but not by *N*-ethylmaleimide, N^G-nitro-L-arginine methyl ester or ruthenium red, which suggests that prostaglandins are involved in the mode of gastroprotective action of **6**.

The genus *Baccharis* is represented in Chile by 48 species.[1] Several of them are used in traditional medicine to protect stomach and liver, restore blood circulation, reduce inflammatory process and cure ulcers, burns and skin wounds.[2] In particular, *Baccharis tola* has been reported to be a producer of acetophenones, coumarines, diterpenoids, triterpenoids and flavonoids.[3,4] In the course of our investigation of medicinal plants from Chile, we report here the gastroprotective effect and cytotoxicity of *ent*-beyer-15-en-18-ol and several *ent*-beyerene derivatives (**2**–**18**). In addition, we discuss the mode of gastroprotective action of **6**, including the involvement of prostaglandins (PGs), nitric oxide (NO), sulfhydryl compounds (SHs) and vanilloid receptors (VR).

From the dichloromethane extract of *B. tola*,[5] *ent*-beyer-15-en-18-ol (**1**) was isolated[6] (Fig. 1). The effect of *ent*-beyer-15-en-18-ol (**1**) on the model of HCl/EtOH-induced gastric lesions[7,8] in mice[9] is shown in Figure 2. An oral administration of **1** at 12.5, 25, 50 and 100 mg/kg (ED_{50} = 50 mg/kg) inhibited the appearance of gastric lesions in a dose-dependent manner compared with the control group (P <0.01). The inhibition displayed by **1** at 50 mg/kg, *p.o.* (53%) was similar to that observed with lansoprazole (57%), while the strongest effect was observed at 100 mg/kg (79%). Taken into account its gastroprotective activity, we decided to prepare lipophilic analogs of **1** (Fig. 1) containing a side chain of fatty acids and cinnamic acids semi synthesized thorough esterification reactions.

Seventeen esters were prepared by standard methods using DCC as coupling agent.[8] In this way, we obtained the compounds **2**-**18**. All proton and carbon resonances were assigned by ^{1}H, ^{13}C, DEPT, HHCOSY, HMQC and HMBC experiments.[10]

Table 1 shows the effect of the semisynthetic derivatives **2**–**18** at 50 mg/kg. The greatest gastroprotective activity was displayed by compounds **2**, and **6**, which resulted as active as lansoprazole at 20 mg/kg and reduced gastric lesions by 68 and 77%, respectively. The gastroprotective activity of compounds **3**–**5** and **8**–**11** did not differ statistically from the control. As for compounds **7** and **12**–**18**, the gastroprotective active were found over the range 24–52%.

In the case of the cinnamic esters **2**–**12**, a significant increase in the gastroprotective activity was observed for compound **6** bearing a chlorine at C-3′ (77%). The effect of chlorine at C-4′ (42%) was similar to that of the parent compound (**1**). Regarding the nitro group (**3**–**5**) and methoxy group (**8**–**10**), the effect was lower than the parent compounds **1**. Furthermore, a significant decrease in the

* Corresponding author. Tel.: +56 02 9787259; fax: +56 02 22713838.
E-mail address: areche@uchile.cl (C. Areche).

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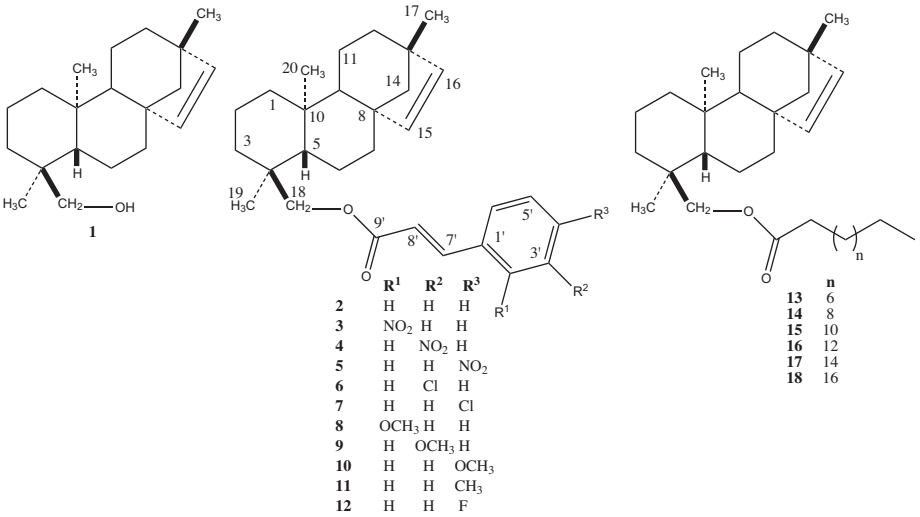

Figure 1. The structures of compounds **2**–**18**.

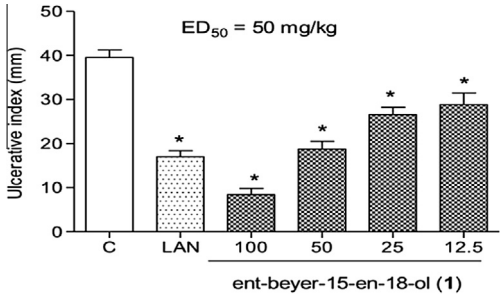

Figure 2. Effects of **1** (12.5, 25, 50 and 100 mg/kg) and lansoprazole (20 mg/kg) on the model of HCl/EtOH-induced gastric lesions in mice. Results are expressed as mean ± SEM, $n = 7$. Analysis of variance followed by Dunnett's test. $^{*}P < 0.01$ compared with control group.

Table 1
Gastroprotective effect of **1** and the semisynthetic derivatives **2**–**18** at 50 mg/kg on HCl/EtOH-induced gastric lesions in mice and cytotoxicity towards AGS cells and human fibroblasts

Compound	*n*	Lesion index (mm)	% lesion reduction	Cytotoxicity IC_{50} (μM)	
				AGS	Fibroblasts
1	7	17.7 ± 1.8*	50*	19 ± 2	31 ± 4
2	7	11.6 ± 1.3*	68	30 ± 3	45 ± 4
3	7	31.0 ± 1.4	14**	25 ± 4	38 ± 5
4	7	35.4 ± 1.7	2**	29 ± 4	44 ± 3
5	7	36.7 ± 2.1	0**	35 ± 5	61 ± 4
6	7	8.4 ± 1.2*	77**	12 ± 2	15 ± 1
7	7	20.8 ± 1.6*	42**	18 ± 1	29 ± 2
8	7	33.4 ± 1.3	7**	151 ± 13	311 ± 9
9	7	34.6 ± 1.8	4**	165 ± 18	289 ± 12
10	7	32.0 ± 1.7	11**	230 ± 11	378 ± 14
11	7	32.9 ± 1.5	8**	39 ± 5	49 ± 6
12	7	27.5 ± 1.0*	24**	58 ± 6	69 ± 4
13	7	17.1 ± 0.9*	52**	21 ± 2	33 ± 3
14	7	17.7 ± 1.6*	51**	33 ± 2	52 ± 4
15	7	19.3 ± 1.2*	46**	38 ± 3	61 ± 5
16	7	19.4 ± 0.6*	46**	64 ± 4	88 ± 6
17	7	20.1 ± 1.1*	44**	228 ± 11	359 ± 15
18	7	23.8 ± 1.0*	34**	321 ± 10	581 ± 13
Lansoprazole	7	12.6 ± 1.5*	65	149 ± 9	291 ± 12
Control	7	35.9 ± 0.9	—	—	—

The results are expressed as mean ± SEM $^{*}P < 0.01$; significantly different compared with the control and $^{**}P < 0.01$ significantly different compared with lansoprazole (ANOVA followed by Dunnett's test), *n* = number of mice.

gastroprotective effect was observed for compounds bearing methyl group **11** and fluorine group **12**.

In the case of the fatty acid esters **13**–**18**, the effect of **13**–**14** and **15**–**16** was similar to that of the parent compound (**1**). No clear relationship was observed between number of carbons in the ester lateral chain and the gastroprotective activity.

The best gastroprotective effect was displayed by **6**, so this compound was chosen for further experiments to explain the possible mode of gastroprotective action. Table 2 shows the effects of **6** on the gastric lesions induced by HCl/EtOH in mice pretreated with Indomethacin (10 mg/kg, s.c.), *N*-ethylmaleimide (NEM, 10 mg/kg, s.c.), N^{G}-nitro-L-arginine methyl ester (L-NAME, 70 mg/kg, ip) or ruthenium red (RR, 3.5 mg/kg, s.c.) at an oral dose of 50 mg/kg.

Endogenous sulfhydryls such as glutathione play an important role in the protection of the gastric mucosa. In this sense glutathione is known to protect the integrity and permeability of the cell membrane and may act as antioxidants, scavengers of free radicals, maintenance of immune function, regulation of protein synthesis and degradation, and the maintenance protein structure.[11,12] In this study, pretreatment with NEM[13] (an SH blocker) have not reduced the gastroprotective activity of **6**, suggesting that the protective effect of this semi-synthetic diterpenoid is not involving the participation of endogenous SHs.

Endogenous PGs are known to be implicated in the mechanism of gastroprotection induced by mild irritants, and necrotizing

Table 2
Effect of *ent-beyer-15-en-18-yl-3-chlorocinnamate* (**6**) on the appearance of gastric lesions induced by HCl/EtOH (*p.o.*) in Indomethacin-, NEM-, L-NAME- and RR-pretreated mice

Treatment	Dose (mg/kg)	Lesion index (mm)
Control	—	42.0 ± 1.9
IND	10	39.9 ± 1.8
NEM	10	41.9 ± 2.1
L-NAME	70	42.7 ± 2.8
RR	3.5	40.5 ± 2.3
6	50	12.1 ± 2.5*
IND + **6**	10 + 50	38.9 ± 2.1
NEM + **6**	10 + 50	15.2 ± 2.1*
L-NAME + **6**	70 + 50	13.1 ± 2.1*
RR + **6**	3.5 + 50	17.0 ± 1.9*
Carbenoxolone	100	13.1 ± 2.9*

Results are expressed as mean ± SEM, $n = 7$. Analysis of variance followed by Dunnett's test. $^{*}P < 0.01$ compared with the respective control.