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Gastric cytoprotective activity of ilicic aldehyde: Structure–activity relationships

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Abstract—A series of sesquiterpene compounds possessing both eudesmane and eremophilane skeletons were tested as gastric cytoprotective agents on male Wistar rats. The presence of an α,β-unsaturated aldehyde on the C-7 side chain together with a hydroxyl group at C-4 is the requirement for the observed antiulcerogenic activity. In an attempt to establish new molecular structural requirements for this gastric cytoprotective activity, a structure–activity study was performed.
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Antitumor, antimicrobial, antifeedant, cytotoxic, antibacterial, antifungal, and allergenic contact dermatitic activities of several sesquiterpene lactones has been previously reported.[1]

Previous studies have determined that dehydroleucodine **1** (Fig. 1), a sesquiterpene lactone of the guaianolide type isolated from *Artemisia douglasiana* Besser, shows a pharmacological cytoprotective effect and significantly prevents the formation of gastric lesion induced by several necrotizing agents. A structure–activity relationship study reveals that the presence of α,β-unsaturated carbonyl groups seems to be responsible for the bioactivity.[2]

It has been reported that gastric cytoprotection may be mediated by at least two different mechanisms. The first one concerning prostaglandins (PG) and the second one involving sulfhydryl-containing compounds of the mucosa. The mechanism of cytoprotection might be mediated, at least in part, by the reaction between

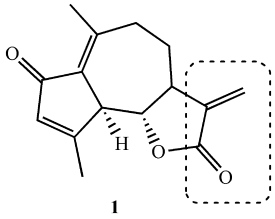

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Figure 1. Structure of dehydroleucodine.

electrophilic acceptor and sulfhydryl-containing compounds of the gastric mucosa.[3] In this regard, α-methylene-γ-lactone and conjugated cyclopentenone are among the most active functional groups.[4]

The main objective of this paper was to investigate the antiulcer activities of several sesquiterpenes (Fig. 2) that were derived from the natural products ilicic acid **2** and ilicic alcohol **3**, both isolated from the aerial parts of *Flourensia oolepis* Blake.[5] In addition to the above-mentioned compounds, the eremophilane tessaric acid **4** was recovered from *Tessaria absinthioides* H. et A. and its derivatives were also tested.[6] Both plant species were grown in the semi-arid central-western region of Argentina. Compounds **2**–**4** were used as starting materials to prepare the derivatives **5**–**14**.

Keywords: Gastric cytoprotection; Sesquiterpenes; Ilicic aldehyde; Eudesmane; Eremophilane.
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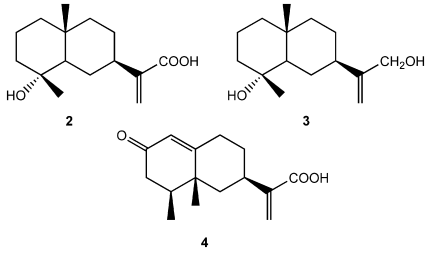


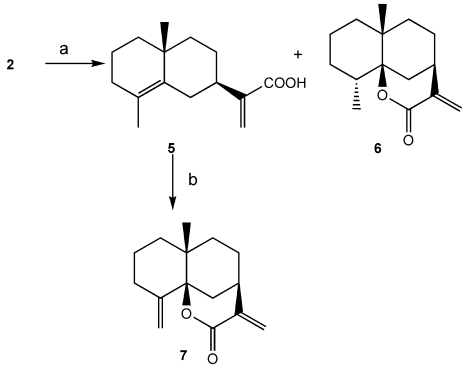
Figure 2. Natural sesquiterpenes from *T. absinthioides* and *F. oolepis*.

Flourensia oolepis Blake was collected at Cuesta del Gato, Juana Koslay, Departamento La Capital, San Luis, Argentina. Its identification was confirmed by Prof. L. A. Del Vitto and a voucher specimen was deposited at the Universidad Nacional de San Luis Herbarium under No. 2329. *T. absinthioides* H. et A. (voucher specimen No. 0461) was collected from El Volcán, Departamento La Capital, San Luis.

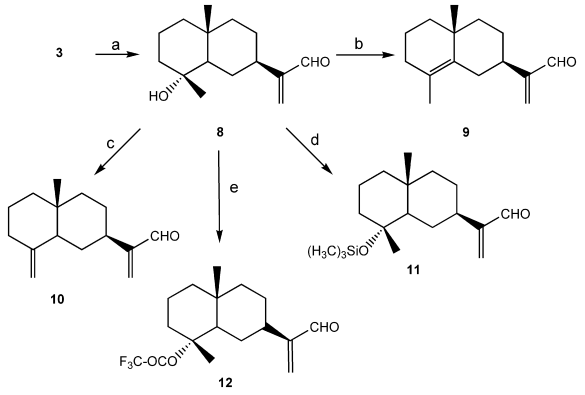
Air-dried aerial parts of *F. oolepis* (2.5 kg dry weight) were extracted, as previously described.[5,7] After several chromatographic purifications, ilicic acid **2** (1.35 g) and ilicic alcohol **3** (1.1 g) were obtained.[7,8] From dried aerial parts of *T. absinthioides* (5 kg dry weight), tessaric acid **4** (25 g) was obtained, as previously reported.[6,9]

As shown in Scheme 1, compounds **5** and **6** were prepared in a one-pot reaction from compound **2**.[10] Photo-oxidation of compound **5** under a sunlight lamp (1000 W) for 40 h with oxygen circulation through a glass frit,[11] followed by triphenylphosphine treatment,[12] led to the formation of compound **7** in 92% yield.[10]

Ilicic aldehyde **8** (Scheme 2) was prepared from alcohol **3** using the Jones reagent in the usual way.[13] After silica gel chromatography (*n*-hexane/EtOAc 1:9 as eluent), the title compound (1.3 g, 87%) was obtained; ^{1}H NMR and ^{13}C NMR are shown in Table 1.[5,14] γ-Costic aldehyde **9**[15] was obtained in good yield by dehydration of **8** with *p*-TsOH in dry C_6H_6. Compound **8** was converted into costic aldehyde **10**,[16] according to a previous method using $POCl_3$ in dry pyridine at −21 °C.[17] Two diverse



Scheme 1. Reagents and conditions: (a) TsOH, C_6H_6, MS 4 Å, 80 °C (after silica gel chromatography 55% **5** and 46% **6**); (b) (i) *i*-Pro, bengal rose, sunlight, 48 h, (ii) MeOH, triphenylphosphine, rt, overnight; (iii) Ac_2O, pyridine, rt, overnight (72%).



Scheme 2. Reagents and conditions: (a) Jones reagent (87%); (b) TsOH, C_6H_6, MS 4 Å, 80 °C (93%); (c) $POCl_3$, pyridine, −20 °C (56%); (d) HMDS, TMSCl, CH_2Cl_2, rt (91%); (e) $(F_3CCO)_2O$, pyridine, DMAP, rt (92%).

chemical transformations were used to obtain **11** and **12**, as shown in Scheme 2.

Compound **13** was prepared from tessaric acid **4** by direct esterification with MeOH/HCl (Scheme 3).[18] The synthesis of 1(10),2,11(13)-eremophilatrien-12-oic acid **14** was carried out by treatment of **13** with $AlCl_3$ and $LiAlH_4$ in THF. This compound was characterized by ^{1}H NMR, ^{13}C NMR, CGMS, and HRMS (Table 1).

Male Wistar rats weighing 200–250 g were used for acute gastric ulcerations induced by ethanol.[19] The animals, randomly assigned into groups (n = 5–8), were deprived of food for 24 h prior to starting the experiments and had free access to water.

Briefly, absolute ethanol (1 mL/animal) was administered intragastrically to control rats and rats pretreated 1 h before with compounds **1**–**14**, respectively (100 mg/kg, suspended in 0.4% carboxymethylcellulose, po). One hour after ethanol administration, the animals were sacrificed and the stomach was removed. The gastric lesions formed were counted and the mean ulcerative index was calculated, according to the method previously described.[2] An index of 0 denoted no erosions, while an index of 5 corresponded to maximal damage. Control rats received absolute ethanol and vehicle (po). A proton pump inhibitor, omeprazole (Ulcozol 10®, Bagó, 20 mg/kg), which is a commonly prescribed drug for increased gastric acid secretion and gastric ulcer, was used as a reference drug for comparison. Omeprazole was also given 1 h before absolute ethanol was administered.

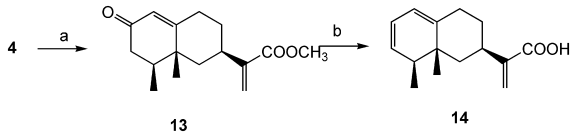
The results are shown in Table 2. Statistical analysis of data was performed using one-way analysis of variance (ANOVA) with Tukey–Kramer multiple comparisons method, with a level of significance of $p < 0.05$.

In previous papers, we have demonstrated the cytoprotective activities of **1** and other related sesquiterpene lactones against the formation of gastric lesions induced by various necrotizing agents.[2,4]

Table 1. ^{1}H (200 MHz), and ^{13}C NMR (50.23 MHz) data of **8**[a] and **14**

H	$8\delta_H$	14 δ_H	C	**8** δ_C		**14** δ_C		^{1}H–^{13}C long range correlation
1	—	5.99 br m	1	20.08*	CH_2	128.82	CH	H-3
2	—	5.53 m	2	25.86*	CH_2	125.62	CH	
2′	—	—	3	44.44*	CH_2	121.41	CH	H-2, H-4
3	—	5.53 m	4	71.21	C	36.07	C	
3′	—	—	5	54.94	CH	36.86	C	H-3, H-4, H-15
4	—	1.65 m	6	76.99	CH_2	39.25	CH_2	
6	1.85 m	—	7	37.28	CH	32.71	CH	
6β	1.26 m	—	8	43.43*	CH_2	32.90*	CH_2	H-13
7	2.53 m	2.73 m	9	40.92*	CH_2	30.85*	CH_2	
12	9.52 m	—	10	34.61	C	143.47	C	H-14
13	6.28 br s	6.34 m	11	154.98	C	144.49	C	H-13
13′	5.97 br s	5.65 m	12	194.70	CH	172.32	C	
14	0.92 s	0.9 m	13	133.03	CH_2	125.40	CH_2	
15	1.11 s	0.88 m	14	18.68	CH_3	20.23	CH_3	
			15	22.43	CH_3	15.12	CH_3	

COLOC correlation of **14** (δ, ppm, TMS, $CDCl_3$).
[a] Colorless needles, mp 83–84 °C. $[\alpha]_D$ −10.6 (*c* 5.7, Me_2CO).
* Interchangeable in each column.



Scheme 3. Reagents and conditions: (a) MeOH/HCl, reflux (92%); (b) $AlCl_3$, $LiAlH_4$, THF, 0 °C (82%).

Table 2. Gastric cytoprotective effect of compounds **1**–**14**

Compound	Ulcerative index[a]	Inhibition of damage[b]
1	0.16 ± 0.10	97
2	4.62 ± 0.12	5
3	4.80 ± 0.12	1
4	4.62 ± 0.12	5
5	4.00 ± 0.31	17
6	2.50 ± 1.00***	48
7	3.37 ± 0.23*	30
8	0.20 ± 0.12***	96
9	4.62 ± 0.12	5
10	3.75 ± 0.28	23
11	2.50 ± 0.28***	48
12 (1° h)	0.66 ± 0.16***	86
12 (2° h)	0.16 ± 0.10***	96
13	4.62 ± 0.37	5
14	3.70 ± 0.28	23
Positive control[c]	3.00 ± 0.28***	38
Control	4.85 ± 0.15	0

[a] All values are means ± SEM.
[b] All values are % of inhibition.
[c] Omeprazole.
* Statistical significance from control at $p < 0.05$.
*** Statistical significance from control at $p < 0.001$.

Pretreatment with omeprazole (20 mg/kg) caused a significant reduction in ethanol-induced necrotic damage of gastric mucosa. Under appropriate conditions, the gastroprotective effect of omeprazole is mainly attributed to an enhancement of the mucus barrier rather than a reduction of acid secretion.[20]

From the present work, results obtained in the ethanol-induced ulcer indicate that ilicic aldehyde **8** gives the highest level of gastric protection from the series (Table 2). The cytoprotective effect of this compound was comparable to that previously reported for dehydroleucodin **1**.[2] Furthermore, compounds **6**–**7** showed statistically significant bioactivity. It should be noted that all the active molecules have an electrophilic unsaturated bond conjugated to the carbonyl group. On the other hand, the carbonyl group must be included in an aldehyde or an α-methylene-δ-lactone functional group. These results are in agreement with those reported for sesquiterpene lactones and structurally related compounds.[2,4]

Although the sesquiterpenes studied here possess similar skeletons, the results indicate that our experimental model responds quite differently to structurally related compounds. Compounds **2** and **3** showing a C-4-α-hydroxyl group and carboxylic acid or hydroxymethylene functional group at the C-7 side chain were inactive. Similar results were observed for derivatives **5**, **9**, and **14**, all of them showing endocyclic unsaturation at ring A of the decaline moiety. Compound **10** possessing an exocyclic C-4 unsaturation displays a very low cytoprotective effect. Similarly, δ-lactones **6** and **7** displayed only a moderate cytoprotective activity.

The above results have suggested that in the eudesmane skeleton an α,β-unsaturated-δ-lactone or α,β-unsaturated aldehyde appeared to play a determinant role in the cytoprotective effect, aside from some discriminating properties of the decaline moiety. This observation has been supported by results obtained when tessaric acid **4**, its methyl ester **13**, and the reduced-dehydrated derivative **14** were assayed. Compounds **4, 13**, and **14** that belong to the eremophilane series did not show an statistically significant activity. In an additional experiment, we found that the inhibition response was dose dependent in the range 25–100 mg/kg (**8** and **9**, $p < 0.01$).

Regarding the influence of the substituents on the flexible side chain and at C-4, an interesting structure–activity correlation can be observed: the presence of an α,

β-unsaturated aldehyde group on the side chain was mandatory for an acceptable cytoprotective activity in this series (compare activities of compounds **2** and **3** with that of **8**). However, the lack of activity obtained for compounds **9** and **10** clearly indicates that the presence of an α,β-unsaturated aldehyde would be a structural requirement but not by itself sufficient for the cytoprotective activity.

To outline the role of this functional group, the trimethylsilyl derivative **11** was prepared. The bioassays carried out with derivative **11** showed a dramatic decrease in activity when compared to that of compound **8** ($p < 0.001$, **8** vs **11**).

Additionally, when the C-4α-hydroxyl group was converted in the corresponding trifluoroacetyl to yield derivative **12**, the bioactivity was similar to that of the parent compound **8**. On account of the good leaving property of trifluoroacetate in compound **12**, its stability under experimental conditions similar to those in the gastric juice was evaluated.[21] In this case, after 1 h of contact with synthetic gastric juice nearly 50% of compound **12** was hydrolyzed to its C-4-hydroxy derivative **8**. It was not possible to detect if the recovered hydroxyaldehyde was a diastereomerically pure compound or a mixture of diastereomers at C-4 (consequence of the solvolysis through an S_N1 mechanism). We perceive that compound **12** might not be active by itself, but perhaps its hydrolyzed derivative **8** (probably as a diastereomeric mixture). Under the same experimental conditions compounds, **8** and **11** proved to be stable after 2 h.

In conclusion, the above-described results are an additional support for our hypothesis suggesting that the polar function at C-4 in compound **8** plays a determinant role in the cytoprotective effect reported here.

The results of the present study proved that in the sesquiterpene series evaluated here, some structural requirements are necessary to elicit cytoprotective effect. Thus, the presence of an electrophilic center (i.e., α,β-unsaturated carbonyl group) together with a second polar group seems to be determinant, whereas conformational requirements from decaline system seem to play only a secondary role.

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8. Ilicic acid **2**, solid powder, mp 181–182 °C, $[\alpha]_D$ −36.1 (*c* 1.53, $CHCl_3$). IR spectra ν_{max} (cm^{-1}): 3420, 2300, 1695, 1625, 945, 895. The ^1H NMR spectra (200 MHz, $CDCl_3$) presented signals at δ 0.93 (s, 3H, H14), δ 1.11 (s, 3H, H15), δ 6.23 (s, 1H, H13), δ 5.63 (s, 1H, H13′), δ 2.50 (s, 1H, H7), δ 1.95 (m, 1H, H6) and δ 1.86 (m, 1H, H6′). Ilicic alcohol 3, solid powder, mp 134–135 °C, $[\alpha]_D$ −43.2 (*c* 1.9, $CHCl_3$). IR spectra ν_{max} (cm^{-1}): 3500–3100, 1170, 1050 (–OH), 1640, 890 ($R_2C{=}CH_2$). The ^1H NMR spectra (200 MHz, $CDCl_3$) presented signals at δ 0.90 (s, 3H, H14), δ 1.10 (s, 3H, H15), δ 2.13 (br s, 1H, HO), δ 4.15 (br s, 2H, H12), δ 4.92 (s, 1H, H13), δ 5.05 (s, 1H, H13′). EIMS [$C_{15}H_{22}O_2$] [M^+, 28] 238, 223, 220, 205, 202, 187, 162, 147, 135.
9. Tessaric acid **4**, white solid, mp 154–155 °C, $[\alpha]_D$ −143.4 (*c* 1.7, $CHCl_3$). UV spectra λ_{max}: 215 nm (ε, 8500) and 243 nm (ε, 14,000). IR spectra 2680, 1695, 1660, 1620, 1600 and 950 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 6.33 (br s, 1H, H13), δ 5.66 (br s, 1H, H13′), δ 5.88 (br s, 1H, H1), δ 0.98 (d, $J = 7$ Hz, 3H, H15), δ 1.10 (s, 3H, H14), δ 2.58 (m, 1H, H7); ^{13}C NMR (50.23 MHz, $CDCl_3$), 125.75, 149.13, 42.04, 35.84, 40.29, 39.43, 32.39, 29.26, 28.86, 173.59, 143.81, 171.64, 125.30, 19.08, 15.35.
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21. Chemical stability of compound **12**. To 50 ml of aqueous solution containing NaCl (34.2 mM) and HCl (84.4 mM) (final pH of 1.2), 150 mg of compound **12** was added. The mixture was stirred at 35 °C, and aliquots of 10 ml were removed every 30 min. Each individual fraction was neutralized with solid $NaHCO_3$ and extracted with Et_2O. The organic layers were dried, evaporated at low temperature, and subjected to analysis by GC–MS. The results showed that the quantitative hydrolysis of compound **12** to **8** was completed after 2 h.