Gastric Anti-ulcer Activity of Several α,β-Unsaturated Carbonyl Compounds in Rats

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Received May 24, 1999; accepted December 24, 1999

The gastric cytoprotective activity of several molecules containing an α,β-unsaturated carbonyl system is reported. We attributed this gastroprotective activity to the presence of a non-hindered Michael acceptor in the molecules assayed and suggested that the mechanism of protection would involve, at least in part, a nucleophilic attack of the sulphydryl group of the gastric mucosa to the β carbon of the Michael acceptors of the compounds assayed.

Key words gastric anti-ulcer activity; gastric protection; Michael acceptor; ethanol-induced injury

We previously demonstrated that the sesquiterpene lactones helenalin and dehydroleucodine significantly prevent the formation of gastric lesions induced by various necrotizing agents.[1] Such cytoprotective activity was also demonstrated in other sesquiterpene lactones having a common feature, a Michael acceptor, in their structure.[1] Furthermore, we reported the gastroprotective activity of the isolated functional groups of this family of natural products. Among them the highest activity is shown by α-methylene-γ-butyrolactone and 2-cyclopenten-1-one, which have an α,β-unsaturated carbonyl system.[2,3] The present study reports the relationship between structure and activity in the gastric cytoprotective effect of several molecules containing an α,β-unsaturated carbonyl system.

MATERIALS AND METHODS

Compounds Utilized 2-Cyclopenten-1-one (**1**), 3-methyl-2-cyclopenten-1-one (**2**), 5,6-dihydro-2*H*-pyran-2-one (**3**), 5,6-dihydro-4-hydroxy-6-methyl-2*H*-pyran-2-one (**4**), *E*-3-furan acrylic acid (**10**), ethyl β-oxo-3-furan propionate (**11**), *E*-cinnamaldehyde (**13**), methyl *E*-cinnamate (**14**), cafeic acid (**15**) and ferulic acid (**16**) were purchased from Aldrich Chemical Company, and cinnamic acid (**12**) was from Merck Co. Piperitenone (**5**) was provided from I. Retamar. Compound **6**, 3-hydroxycarvotagenone, was obtained as a minor constituent from *Artemisia douglasiana*.[4] Compound **7**, 3-acetoxycarvotagenone was prepared from **6** as follows: to 3-hydroxycarvotagenone (30 mg) dissolved in dry pyridine (1 ml) was added Ac_2O (1 ml) and the mixture was allowed to stand overnight at room temperature. The reaction solution was poured into ice-H_2O and extracted with Et_2O. The organic layer was washed with sat. $NaHCO_3$ and H_2O successively, then was evaporated to give a crude product, which was purified by column chromatography (silica gel) to give **7** (18 mg), which was identified by spectroscopic data (^{1}H-NMR, ^{13}C-NMR and IR). Aldehyde I (**8**) and aldehyde II (**9**) were prepared according to the procedure previously reported.[5]

Induction of Gastric Lesions Gastric lesions were produced according to the method of Robert *et al.*[6] Wistar rats weighing 200—220 g were fasted for 24 h and deprived of water for 19 h prior to the experiments. All rats were housed in wire mesh-bottomed cages throughout the study to prevent coprophagy. Absolute ethanol (1 ml) administered orally was employed as the necrotizing agent, and 1 h later the animals were decapitated. The stomachs were removed, opened along the greater curvature, and washed gently with ice-cold saline solution. The degree of erosion in the glandular part of the stomach was assessed from a scoring system designed by Marazzi-Uberti and Turba[7] from 0 (no erosions) to 5 (maximal damage). The results were expressed in terms of an ulcer index which is the average severity of erosions per rat for each group (5—7 animals). The sum of these values was divided by the number of animals. Compounds **1**—**16** were prepared just before the experiment (40 mg/kg), suspended in 0.4% carboxymethyl cellulose (CMC) and were given 60 min prior to the necrotizing agent. The control rats were given 1 ml absolute ethanol (*p.o.*).

Statistics The statistical significance of difference among means was assessed by Student's *t*-test. Differences *vs.* control (EtOH) were considered significant at $p<0.05$. In Table 1, all data are presented as the average of 5—7 animals.

RESULTS AND DISCUSSION

The cytoprotective activity of **1** against gastric ulcer induced by absolute ethanol was 1.3 (see Table 1), indicating 73% inhibition of damage. Compound **2**, in contrast, showed a low level of cytoprotective activity of about 4.3. This result confirms our previous hypothesis about the lack of activity shown by sterically hindered Michael acceptors. Similarly, **3** showed 88% inhibition of damage, and activity of molecule **4** is lower than that of **3**. The low level of effect shown by **4** and **5** can be attributed to a problem of steric hindrance. The α-methyl substituted compound **6** shows a moderate but significant cytoprotective activity ($p<0.05$ *vs.* control). When **6** was acetylated to give the derivative **7**, the bioactivity was increased. Other active compounds were molecules **8** and **9**. It should be noted that these compounds have an electrophilic unsaturated bond conjugated with a carbonyl group. Among **10** to **16**, only the α,β-unsaturated ester methyl cinnamate (**14**) showed moderated gastroprotective activity.

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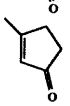
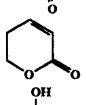
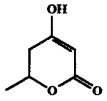
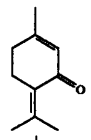
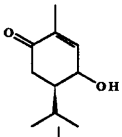
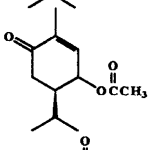
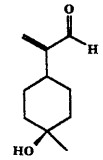
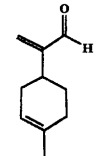
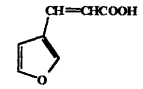
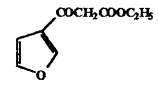
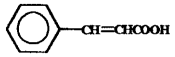
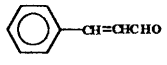
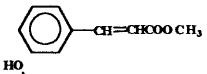
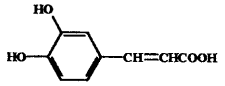
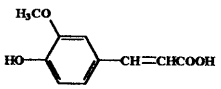
Table 1. Gastroprotection of Gastric Mucosa against EtOH-Induced Damage of α,β-Unsaturated Carbonyl Compounds in Rats

Compound			Ulcer index Mean±S.E.M.	Inhibition (%)
No.	Name	Structure		
Control			4.75±0.15	—
1	2-Cyclopenten-1-one		1.30±0.40*	73
2	3-Methyl-2-cyclopenten-1-one		4.30±0.40	9
3	5,6-Dihydro-2*H*-pyran-2-one		0.55±0.05*	88
4	5,6-Dihydro-4-hydroxy-6-methyl-2*H*-pyran-2-one		4.66±0.57	2
5	Piperitenone		4.30±0.80	9
6	3-Hydroxycarvotagenone		3.60±0.49*	24
7	3-Acetoxycarvotagenone		1.50±0.55*	68
8	Aldehyde I		0.70±0.16	85
9	Aldehyde II		0.70±0.16*	85
10	β-(3-Furyl)acrylic acid		4.60±0.30	3
11	β-Oxo-3-furan-ethylpropionate		4.75±0.50	0
12	Cinnamic acid		5.00±0.01	0
13	Cinnamaldehyde		4.50±0.01	5
14	Methyl cinnamate		2.90±0.01*	39
15	Cafeic acid		5.00±0.01	0
16	Ferulic acid		4.87±0.12	0

CMC+EtOH served as the control. Asterisks denote significant differences from the control ($p<0.05$; Student's t-test). All values were expressed as mean±S.E.M.

These results appear to confirm that the presence of a non-hindered α,β-unsaturated carbonyl group seems to be an essential structural requirement for the gastric cytoprotective activity of these compounds. We focused our attention on the possible involvement of sulfhydryl-containing groups of the gastric mucosa (reduced glutathione and others) as mediators in the process of cytoprotection.[8] Vasoprotection seems to be the common mechanism of gastroprotection by sulfhydryl-related compounds because they decrease the chemically-induced vascular damage and increase the gastric mucosal blood flow.[9] The reduction in cytoprotective activity of several related compounds produced by *N*-ethylmaleimide[10] suggests, together with the aforementioned observations, a possible mechanism of cytoprotection by the Michael acceptors containing the compounds studied. The proposed mechanism would involve a nucleophilic attack of the sulfhydryl group to the β carbon of the Michael acceptors of the compounds assayed. However, caution is required in such interpretation and additional analysis will be necessary to confirm this speculation.

Acknowledgements The authors are grateful to Ing. Juan Retamar (IPNAYS, U.N. del Litoral, Argentina) for supplying **5**. This work was supported by grants from the Consejo Nacional de Investigaciones Cíentificas y Técnicas (CONICET) and Universidad Nacional de San Luis (CYTED).

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