Gastric Cytoprotective Activity of 2-Cyclopenten-1-one and Related Compounds

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The cytoprotective activity of the isolated functional groups of several sesquiterpene lactones is reported. Among them the highest activity is shown by α-methylen-γ-butyrolactone and 2-cyclopenten-1-one. The activity shown by those Michael acceptors with a β carbon hindered by an alkyl substituent was always lower or almost null. A three-way mechanism of action is proposed: a) reduced glutathione synthesis, b) prostaglandin synthesis and c) mucosal glycoprotein synthesis.

Key words cytoprotection; Michael acceptor; reduced glutathione

We have previously demonstrated that the sesquiterpene lactones Helenalin (**1**) and Dehydroleucodin (**2**) significantly prevent the formation of gastric lesions induced by various necrotizing agents. Such cytoprotective activity was also demonstrated in other sesquiterpene lactones having a common feature, a Michael acceptor, in their structure.[1] Thus, we suggested that the pharmacological activity of this family of natural products is related to the presence of an α-methylene-γ-butyrolactone (**3**) system.[2]

In view of the structural nature of the potentially active groups present in these compounds, we focused our attention on the possible involvement of SH-containing groups of the mucosa as mediators in the process of cytoprotection.[3] The proposed mechanism would involve a nucleophilic attack of the -SH group to the β carbon of the Michael acceptors of the compounds assayed (**1**—**9**). The previously studied compounds show two common features in their structure, an α-methylen-γ-butyrolactone and an α, β unsaturated cyclopentenone system. These two functional groups can react, in principle, as Michael acceptors with the SH containing groups of the mucosa (reduced glutathione (GSH) and others).

In the present study we report a relationship between structure and activity in the gastric cytoprotective action of the isolated **3**, α, β unsaturated cyclopentenone and other related compounds in rats.

RESULTS AND DISCUSSION

First, we demonstrated the cytoprotective activity of **1** and **2** and other related sesquiterpene lactones against the formation of gastric lesions induced by various necrotizing agents.[1] Later, we attributed this cytoprotective activity to the presence of a non-hindered Michael acceptor in the molecules assayed and suggested that the mechanism of protection would be, at least in part, mediated through a nucleophilic reaction between the Michael acceptors and the sulfhydril containing groups of the mucosa. Since the mechanism of the Michael addition is influenced by pH and, in view of the pK_a's of alkyl thiols and of the conjugated acid enones which are 10 and −5, respectively, it is likely that the reaction proceeds *via* a neutral Michael acceptor and deprotonated thiol under basic and neutral conditions, while under acidic conditions, a mechanism involving a protonated Michael acceptor and a neutral thiol is more reasonable. While the rumen of the stomach is strongly acidic, the putative Michael addition occurs in the mucosa where the pH is closer to neutral. Hence, the reaction probably proceeds *via* deprotonated thiol. In these studies were demonstrated that the activity of these molecules was due to the presence of the **3** and 2-cyclopenten-1-one (**4**) groups. On the other hand, the 3-methyl-2-cyclopenten-1-one (**5**) group present in **2** showed almost no activity. This was attributed to the steric hindrance produced by the methyl group on C-3 of **2**. In a recent study (unpublished observations), we observed a high level of activity for the isolated molecule **3**. This last discovery demonstrated that the cytoprotective activity is not related to the guaianolide or pseudoguaianolide frame, but to the presence of a non-hindered Michael acceptor. We have now undertaken a comparative study of the activity of the isolated **4** and **5** molecules. This was done not only in view of the different level of activity exhibited by these two functional groups as part of a sesquiterpene lactone frame, but most importantly, because this kind of functional group belongs also to some prostaglandin frames (PG A2 and I).

The cytoprotective activity of **4** against gastric ulcers induced by absolute ethanol was of 1.3 in the scale developed by Marazzi-Uberti and Turba.[4] A similar level of activity was also shown by this functional group as a part of **1**. Compound **5**, instead, showed a low level of cytoprotective activity of about 4.3. This result is in agreement with the values observed for this functional group as part of **2**. This result confirms our previous hypothesis about the lack of activity shown by sterically hindered Michael acceptors. This result also confirms the presence of two active Michael acceptors in **1** and only one in **2**. The activity of molecules **4** and **5** resulted lowr than that of **3**. This result shows **3** to be the best Michael acceptor assayed for our group for this kind of reaction. In order to extend our work to other Michael acceptors, we evaluated the activity of **6**—**9** (Table 1). The low level of activity shown by **8** and **9** can be attributed to a problem of steric hindrance; however, we can not explain why **6** and **7** also show a low level of

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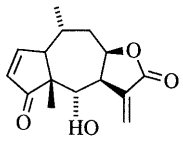
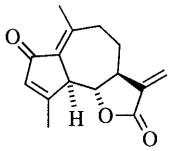
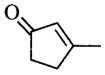
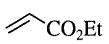
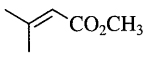
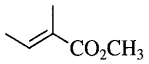
activity. The mechanism of action of these compounds could be related to an incremental change in the biosynthesis of GSH. The increment in the amount of cellular GSH produced by PG A2 and I2 (both of them having an α,β-cyclopetenone ring) has been previously reported.[5] In the same paper, a similar activity for different cyclopentenone derivatives is also reported. Finally, they suggest that an α,β-unsaturated carbonyl moiety is responsible for enhancing the biosynthesis of glutamylcysteinesynthetase and GSH in cultured cells. On the other hand, this endogeneous GSH has been suggested to have ulcer-preventing effects in gastric tissue exposed to a variety of irritants *in vivo*,[6-8] and to have protective effects in cultured gastric mucosal cells exposed to absolute ethanol[9] and acid.[10] Besides this, according to Minamata *et al.*,[11] endogeneous GSH would regulate PG synthesis; thus, at a higher GSH concentration, PGE2 synthesis was remarkably enhanced.[12,13] PG's are also known to be cytoprotective agents against the development of ulcers; however, the mechanism by which PG prevents the formation of these ulcers remains unknown, although it seems to be related to the synthesis of mucosal glycoproteins. The incremental change in the synthesis of mucosal glycoproteins produced by **2** has been recently reported by our group.[14]

The reduction in cytoprotective activity of these compounds produced by the action of indomethacin and *N*-ethylmaleimide[14] suggests, together with the aforementioned observations, the following as a possible general mechanism of cytoprotection by the Michael acceptors containing compounds studied in our research group: (see Chart 1).

Table 1. Protection of Gastric Mucosa against Et-OH-Induced Damage of 2-Cyclopenten-1-one and Related Compounds

Name of compound	Chemical structure	Cytoprotective effect (u.i.)
Helenalin **1**		0.33±0.18* (n=10)
Dehydroleucodin **2**		0.25±0.15* (n=15)
α-Methylen-γ-butyrolactone **3**		0.10±0.08* (n=12)
2-Cyclopenten-1-one **4**		1.30±0.40* (n=10)
3-Methyl-2-cyclopenten-1-one **5**		4.30±(NS)0.40* (n=10)
Ethyl acrylate **6**		4.30±(NS)0.50* (n=10)
Acrylonitrile **7**		3.50±(NS)0.50* (n=10)
8,3-Dimethylacrylic acid methyl ester **8**		4.50±(NS)0.80* (n=10)
Tiglic acid methyl ester **9**		3.30±(NS)0.50* (n=10)
CMC+EtOH **10**		4.75±0.15 (n=10)

CMC (carboxymethyl cellulose)+EtOH served as the control. Number of animals given in parentheses. Asterisks denote significant differences from the control ($p<0.005$; NS, not significant). All values were expressed as mean±S.E.M. Statistical analysis was carried out by an unpaired Student's t-test.

MATERIALS AND METHODS

Compounds Utilized Compounds **4**, **5**, ethylacrylate (**6**), and acrylonitrile (**7**) were purchased from Aldrich Chemical Company, and **3** was from Sigma Chemical Company. Methyl esters of 3,3-dimethyl acrylic acid (**8**) and tiglic acid (**9**) were prepared by the usual treatment with diazomethane and further purification by column chromatography on Silica gel 60. Mixtures of hexanes: ethyl acetate of increasing polarity was used as the eluent. The natural products **1** and **2** were isolated and identified as reported in.[1] Compounds **1** and **2** were used as controls.

General Procedures The purity and identity of all compounds used in the present study were checked according to physical and spectral properties. Melting points were taken on a Leitz hot-stage apparatus; analytical TLC was performed on 0.25 mm silica gel precoated plates with fluorescent indicator UV 254. ^{13}C-NMR and ^{1}H-NMR were recorded on a Brucker EM-200 spectrometer in $CDCl_3$ and tetramethylsilane (TMS) was used as the internal standard. IR spectra were recorded on a Beckman IR-10.

Induction of Gastric Lesions Gastric mucosal damage was induced by absolute EtOH (1 ml/rat, v.o.), according to Robert, *et al.*[15] The nine compounds or the vehicle were given intragastrically 60 min prior to the administration of EtOH, as indicated in Table 1. Animals were

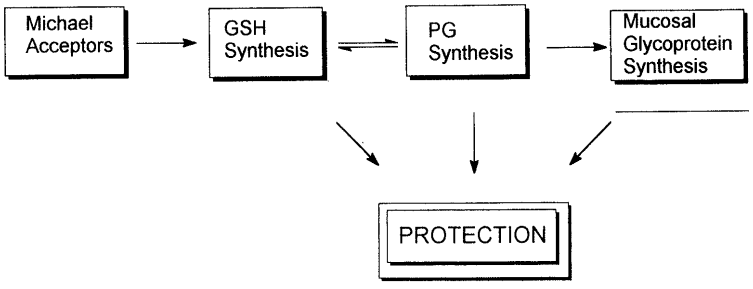


Chart 1. Three-Way Mechanism of Action of Michael Acceptors

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sacrificied 60 min after the application of EtOH. The stomach was removed and opened along the greater curvature. The degree of erosion in the glandular part of the stomach was assessed from a scoring system designed by Marazzi-Uberti and Turba[4] as follows:

0, no erosions; 1, 1—3 small erosions; 2, more than 3 small erosions or one large erosion; 3, one large erosion and more than 3 small erosions; 4, 3—4 large erosions; 5, more than 3—4 large erosions and/or ulcer perforation. The results were expressed in terms of an ulcer factor (UF), which is the sum of animals with an average severity of erosions per rat for each group on a scale from 0 to 5. All values were expressed as the mean ± S.E.M. Statistical analysis was carried out with an unpaired Studient's test.

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