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THE GASTRIC CYTOPROTECTIVE EFFECT OF SEVERAL SESQUITERPENE LACTONES

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ABSTRACT.—The aerial part of *Artemisia douglasiana*, used in folk medicine as a cytoprotective agent against the development of peptic ulcer, was studied, its active principle dehydroleucodine [**1**] isolated, and its pharmacological properties analyzed. In order to establish whether or not the reported activity is particular to sesquiterpene lactones, the study of the cytoprotective activity of several related guaianolides and pseudoguaianolides from plants was undertaken. Ludartin [**3**], 8-angeloyloxy-3-hydroxyguaia-3(15),10(14),11(13)-trien-6,12-olide [**4**], hymenin [**5**],mexicanin I [**6**], helenanin [**7**], and 9-*O*-desacetylspathulin-2-*O*-angelate [**8**] were found to exhibit protection. Desacetoxymatricarin [**2**] did not show cytoprotective activity. The results obtained from the different sesquiterpene lactones studied suggest that the presence of the α-methylene-γ-lactone moiety is, in principle, a requirement for the observed antiulcerogenic activity.

Artemisia douglasiana Besser is a hexaploid species whose origin was attributed by Keck (1) as a hybrid between *Artemisia suksdorfii* Piper and *Artemisia ludoviciana* Nutt. It is found on the western slopes of the Rockies and in northern Baja California. The first report of its occurrence in Argentina appeared in 1967. In that year Ariza Espinar (2) reported the presence of *A. douglasiana* as an adventitious plant in San Juan and Mendoza provinces. The oldest Argentinian herbarium material dates from 1934. Probably *A. douglasiana* was introduced into Argentina from Chile[1].

Phytochemical studies showed *A. douglasiana* as a species with marked disparity in secondary metabolite composition (3–6).

In Argentina *A. douglasiana* is used in folk medicine and known by the common name of "matico" (2). The popular use of the infusion of the boiled leaves of matico as a cytoprotective agent against peptic ulcer and the external treatment of sores and ulcers prompted us to carry out preliminary pharmacological experiments with the aqueous extract of the aerial parts of matico to evaluate whether it showed the aforementioned properties. In fact, in preliminary evaluations carried out in our laboratory the aqueous extract showed reproducible cytoprotective activity against ulcerogenic agents such as absolute EtOH in rats.

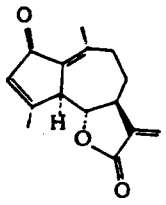
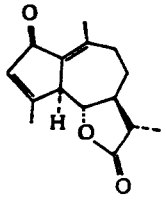
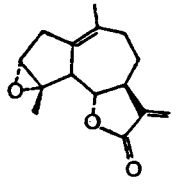
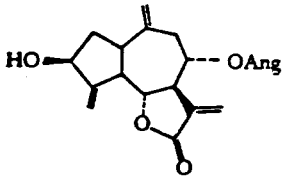
The results of this test prompted us to undertake a phytochemical study of the plant in order to isolate the active cytoprotective principle, dehydroleucodin [**1**], a sesquiterpene lactone of the guaianolide type. In addition, in order to establish whether cytoprotective activity is particular to sesquiterpene lactones, the cytoprotective activity of several related guaianolides and pseudoguaianolides was also evaluated. Table 1 shows the sesquiterpene lactones studied, their chemical names, and the plant material from which they were obtained.

EXPERIMENTAL

PLANT MATERIAL.—*A. douglasiana*, *Artemisia mendozana* DC. var. *paramilloensis*, *Parthenium hys-*

[1]L. Del Vitto, Herbario de la Universidad Nacional de San Luis, personal communication.

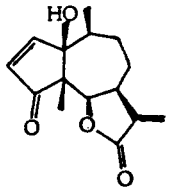
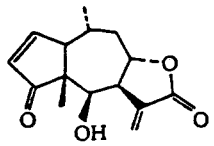
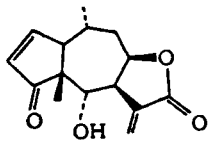
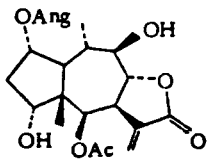
TABLE 1. Cytoprotective Effect of Sesquiterpene Lactones at an Oral Dose of 40 mg/kg.

	Sesquiterpene Lactone	Plant Material	Cytoprotective Effect[a]
GUAIANOLIDES	**1** Dehydroleucodin 	*Artemisia douglasiana*	0.25
	2 Desacetoxymatricarin 	*Artemisia mendozana* var. *paramilloensis*	5
	3 Ludartin 	*Stevia yaconensis* var. *subeglandulosa*	0.5
	4 8-Angeloyloxy-3-hydroxyguaia-3(15), 10(14),11(13)-trien-6,12-olide 	*Vernonia nitidula*	0.33

terophorus L., *Gaillardia megapotamica* (Spreng.) Baker var. *scabiosoides* (Arn.) Baker, and *Helenium alternifolius* (Spreng.) Cabrera were all collected in San Luis, Argentina, and a voucher specimen of each one was deposited in the Herbarium de la Universidad Nacional de San Luis, numbered 55, 1443, 1672, 2862, and 2841, respectively.

The collection and classification of *Stevia yaconensis* var. *subeglandulosa* Hiron and *Vernonia nitidula* are described in Sosa *et al.* (7) and Bardón *et al.* (8), respectively.

TABLE 1. Continued.

	Sesquiterpene Lactone	Plant Material	Cytoprotective Effect[a]
PSEUDOGUAIANOLIDES	**5** Hymenin 	*Parthenium hysterophorus*	0.25
	6 Mexicanin I 	*Gaillardia megapotamica* var. *scabiosoides*	0.25
	7 Helenalin 	*Gaillardia megapotamica* var. *scabiosoides*	0.33
	8 9-*O*-Desacetylspathulin-2-*O*-angelate 	*Helenium alternifolius*	0.33

[a]0, no erosions; 1, 1–3 small erosions (4 mm diameter or smaller); 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, any very large erosion or ulcer perforation.

EXTRACTION AND ISOLATION.—The air-dried material was soaked in $CHCl_3$ at room temperature (3 × 48 h). The combined $CHCl_3$ extracts were evaporated in vacuo and dissolved in 95% EtOH, and 4% aqueous lead tetraacetate solution was added. The aqueous cloudy solution was filtered through a celite pad, and the filtrate was concentrated under vacuum. The mixture was extracted three times with $CHCl_3$,

and the solution was concentrated under vacuum. The residue obtained was chromatographed in a medium pressure chromatography system. Different mixtures of EtOAc and hexanes were used as eluents.

The procedures of extraction of *S. yaconensis* and *V. nitidula* are described in Sosa *et al.* (7) and Bardón *et al.* (8), respectively.

CHARACTERIZATION OF THE NATURAL PRODUCTS.—Nmr, ir, uv, mp, $[\alpha]_D$, and ms data of dehydroleucodin [**1**] isolated from *A. douglasiana*, desacetoxymatricarin [**2**] isolated from *A. mendozana*, hymenin [**5**] isolated from *P. hysterophorus*, mexicanin I [**6**] and helenalin [**7**] isolated from *G. megapotamica*, and 9-*O*-desacetylspathulin-2-*O*-angelate [**8**] isolated from *H. alternifolius* were all in agreement with previously reported data (9–14).

Ludartin [**3**] isolated from *S. yaconensis* and 8-angeloyloxy-3-hydroxyguaia-3(15),10(14),11(13)-trien-6,12-olide [**4**] isolated from *V. nitidula* were identified by Sosa *et al.* (7) and Bardón *et al.* (8), respectively.

INDUCTION OF GASTRIC LESIONS.—Gastric lesions were produced according to the method of Robert *et al.* (15). Male Wistar rats weighing ca. 180 g were fasted for 24 h and deprived of H_2O for 19 h prior to the experiments. All rats were housed in wire-mesh-bottom cages throughout the study to prevent coprophagy. Absolute EtOH (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 stomach was assessed from a scoring system designed by Merazzi-Uberti and Turba (16), as follows: 0, no erosions; 1, 1–3 small erosions (4 mm diameter or smaller); 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, any very large erosion or ulcer perforation.

The results were expressed in terms of an ulcer factor which is the average severity of erosions per rat for each group on the scale from 0 to 5. The sum of these values was divided by the number of animals. The drugs tested in this study were prepared just before the experiment as follows: Compounds **1–8** and indomethacin were suspended in 0.4% carboxymethyl cellulose (CMC). The control rats (group I) were given 1 ml absolute EtOH. Compounds **1–8** (40 mg/kg) were given 60 min before the oral administration of EtOH to group II. The experiments with indomethacin and **1** were carried out as follows: The former was administered 60 min before the administration of **1** and 2 h before the administration of absolute EtOH (Figure 1). Experiments to determine the relationship between dose and response of cytoprotective activity of **1** against gastric lesions induced by EtOH were carried out in four groups of rats. Compound **1** was given in increasing doses of 3, 10, 20, and 40 mg/kg 60 min before the oral administration of EtOH (Figure 2).

DISCUSSION

The $CHCl_3$ extract of the air-dried aerial parts of *A. douglasiana* showed significant cytoprotective activity. The chromatographic purification of the extract yielded almost exclusively **1** (8 g/kg), a known sesquiterpene lactone of the guaianolide type. To our knowledge **1** has not been previously reported as a constituent of *A. douglasiana*.

The pharmacological tests of **1** in rats were undertaken in order to check if **1** was the compound responsible for the antiulcerogenic activity exhibited by the crude extract. When the absolute EtOH was orally administered to rats, hemorrhagic lesions that occurred mostly in the corpus were observed. Usually, 15–20 lesions could be counted. No gross lesions were developed in the forestomach.

We demonstrate in the present paper that **1** significantly prevented the formation of gastric lesions induced by EtOH (Figure 1) and inhibited dose-dependently the formation of erosions (Figure 2). We also showed that **1** prevented gastric damage produced by 0.6 N HCl, 0.2 NaOH, and 25% NaCl.

Although **1** is effective against EtOH-induced ulcers when the vehicle H_2O does not contain CMC, more uniform results were obtained when CMC (0.4%) was employed. CMC (0.4%) solutions did not show cytoprotective activity. CMC (0.4%) has been employed in similar procedures in order to suspend cytoprotective gastric substances (17).

A recent light and transmission electron microscopic study has confirmed that **1** pretreatment prevents the gross hemorrhagic lesions produced by EtOH.[2]

[2]R. Piezzi and J.A. Guman, unpublished observations.

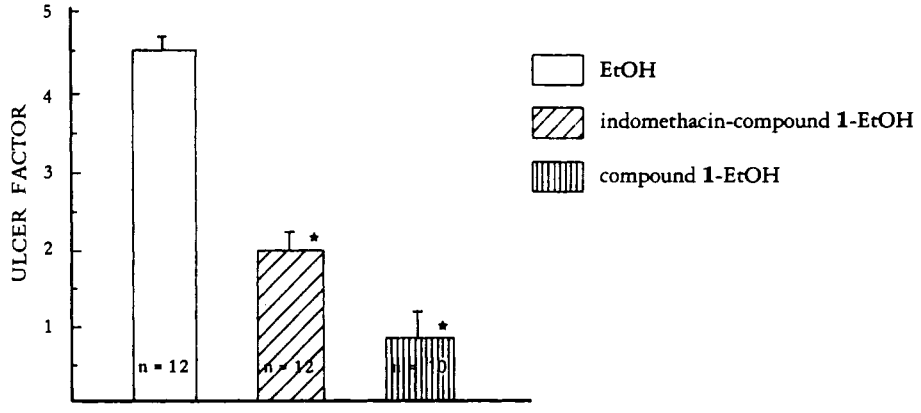


FIGURE 1. Effects of oral dehydroleucodine [**1**] and indomethacin on EtOH-induced gastric ulcers in the rat.
★ $P<0.05$, vs control (Student's t-test)
n = number of animals.

Moreover, by other histological examinations, gastric tissue slices of rat stomach were stained with periodic acid-Schiff (PAS). Pretreatment with **1** 60 min before EtOH administration prevented the reduction of PAS-positive substances (gastric mucopolysaccharides).[3]

Recently, prostaglandins (PG) and PG derivatives have been shown to prevent the formation of ulcers by a mechanism independent of their antisecretory properties (18,19) and to protect the gastric mucosa against lesions induced by various necrotizing agents (15). In order to examine the possible involvement of endogenous PG in the protective action of **1**, the rats were treated with indomethacin (5 mg/kg) 60 min before the administration of **1** (40 mg/kg). The results indicate that indomethacin pretreatment resulted in a significant reduction of the cytoprotective action of **1** (Figure 1). In the conditions used in our experiments, indomethacin did not produce lesions (ulcer

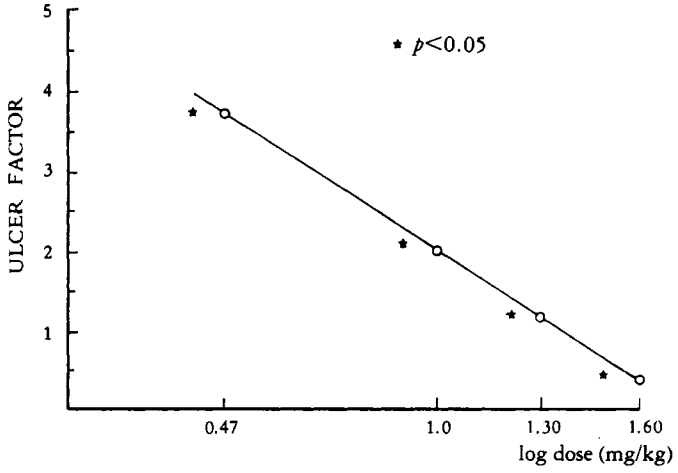


FIGURE 2. The log dose-effect relationship of cytoprotective activity of dehydroleucodine [**1**].

[3]L. Scardapane, S. Dominguez, and R. Piezzi, unpublished observations.

factor = 0). Other authors have reported similar results by using the same dose and time (17). Hollander *et al.* (20) found that sucralfate (a drug used in the treatment of gastric ulcerations) is capable of protecting gastric mucosa against EtOH-induced damage by releasing endogenous PG.

Szabo (21) stressed that gastric cytoprotection might be mediated through at least two different mechanisms, one concerning PG, and the other involving SH-containing compounds of the mucosa. $Al(OH)_3$ would be one of the first compounds which exhibits its protective effect via both pathways, namely PG- and SH-containing compounds (22).

The mechanism of the protective action of **1** remains unknown but seems to be related to endogenous PG. Our work is focused now on the confirmation of the mechanism of action of **1**.

On the other hand, in order to establish whether or not the reported activity was particular to guaianolides, the study of the gastric cytoprotective activity of related guaianolides was undertaken.

Assays of desacetoxymatricarin [**2**], a guaianolide recently isolated in our laboratory from *A. mendozana*, showed no cytoprotective activity against chemically induced ulcers in rats, even when the dose checked was of 40 mg/kg. On the other hand, ludartin [**3**] a guaianolide isolated from *S. yaconensis*, and 8-angeloyloxy-3-hydroxyguaia-3(15),10(14),11(13)-trien-6,12-olide [**4**] isolated from *V. nitidula* showed almost the same activity exhibited by **1** in doses of 40 mg/kg.

Compounds **1**, **2**, and **3** may be viewed as model compounds, with β-substituted cyclopentenone and α-methylene-γ-lactone in **1**, β-substituted cyclopentenone and α-methyl-γ-lactone in **2**, and α-methylene-γ-lactone in **3** (Table 1).

In view of the results obtained with **1**, **2**, and **3**, it seems that the presence of an exocyclic methylene group conjugated to a γ lactone would be a structural requirement needed for the biological activity exhibited for these compounds.

This was further tested by assays of some related pseudoguaianolides. The first pseudoguaianolide checked was hymenin [**5**] isolated in our laboratory from *P. hysterophorus*. The prevention by **5** of gastric lesions produced by EtOH exhibited a dose-dependent variation similar to that exhibited by **1** (Figure 2). Mexicalin I [**6**], a pseudoguaianolide isolated in our laboratory from *G. megapotamica*, helenanin [**7**], an pseudoguaianolide also isolated from *G. megapotamica* but differing from **6** in the stereochemistry at C-6 and C-8, and 9-*O*-desacetylspathulin-2-*O*-angelate [**8**] isolated from *H. alternifolius* acted like **5**, inhibiting the development of gastric mucosal damages at doses of 40 mg/kg.

All the pseudoguaianolides examined showing antiulcerogenic activity have both the β-unsubstituted cyclopentenone ring and the α-methylene-γ-lactone functionality. Compound **8** possesses the α-methylene-γ-lactone moiety exclusively.

In view of the results reached in this preliminary laboratory scale investigation we conclude that: (a) the presence of the α-methylene-γ-lactone moiety is a requirement for the cytoprotective activity observed, and (b) the presence of the β-substituted or -unsubstituted cyclopentenone ring is not a structural requirement for cytoprotective activity contrary to its requirement for antitumor (23), antimicrobial (24), and antifeedant (25) properties.

Currently a number of additional sesquiterpene lactones as well as other natural and synthetic products are being investigated.

Although some reports about the biological activity of sesquiterpene lactones have described them as antitumor, antimicrobial, antifeedant, cytotoxic, antibacterial, antifungal, allergenic contact dermatitic, and plant growth regulatory compounds (23–

27), this is the first study reporting them as cytoprotective compounds against peptic ulcers chemically induced by EtOH.

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