Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Ethnopharmacology 100 (2005) 260–267
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Anti-ulcerogenic activity of xanthanolide sesquiterpenes from *Xanthium cavanillesii* in rats

Laura S. Favier[a], Alejandra O.M. María[b], Graciela H. Wendel[b], Eduardo J. Borkowski[a], Oscar S. Giordano[a], Lilian Pelzer[b], Carlos E. Tonn[a,*]

[a] *INTEQUI-CONICET-UNSL, Departamento de Química, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Chacabuco y Pedernera, 5700 San Luis, Argentina*
[b] *Unidad de Farmacología, Departamento de Farmacia, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Chacabuco y Pedernera, 5700 San Luis, Argentina*

Received 20 December 2004; received in revised form 26 January 2005; accepted 17 February 2005
Available online 17 May 2005

Abstract

The preventive effect of natural xanthanolides as well as a series of synthetic derivatives on ulcer formation induced by absolute ethanol in rats was examined. Among the compounds tested, xanthatin gave the strongest protective activity. The inhibitory action exerted by this molecule on the lesions induced by 0.6N HCl and 0.2N NaOH was highly significant, reducing ulceration in the range of 58–96% at a dose from 12.5 to 100 mg/kg. 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. In order to explore this possibility, a theoretical conformational analysis was performed. We suggest that the mechanism of protection would involve, at least in part, a nucleophylic attack of the sulfhydryl group from the biological molecules present in the gastric mucosa to electrophylic carbons accessible in suitable Michael acceptors.

Keywords: Xanthanolides; Xanthatin; Gastric anti-ulcer activity; Gastric; *Xanthium cavanillesii*

1. Introduction

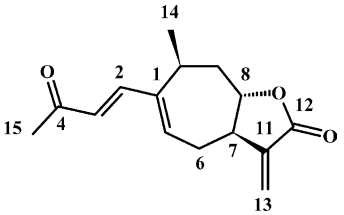
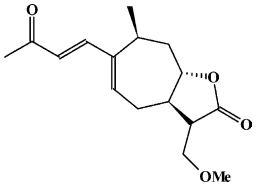
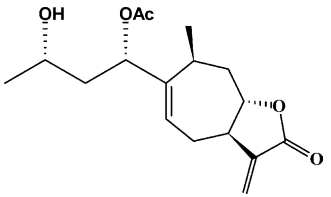
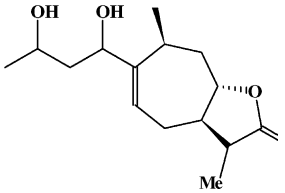
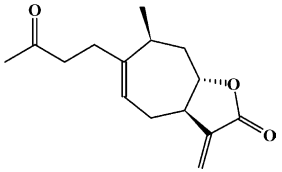
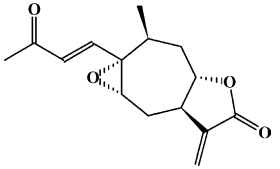
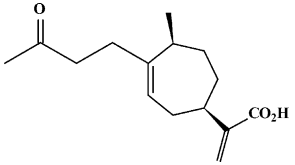
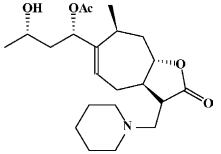
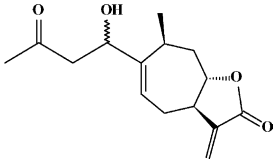
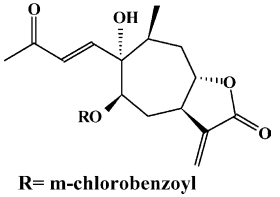
The genus *Xanthium* (Asteraceae, Heliantheae) is represented by a relatively limited number of species distributed in nearly all parts of the world. The chemistry of this genus is very uniform and sesquiterpenes having xanthanolide skeleton have been reported in all phytochemical studies (Riscala et al., 1994). Some *Xanthium* species are reputed in the treatment of fever, leucoderma, and herpes (Saxena and Mondal, 1994). On the other hand, xanthanolide sesquiterpenes have shown several biological activities such as anti-bacterial toward methicillin-resistant *Staphylococcus aureus* (Sato et al., 1997), and cytotoxic activity toward human cancer cell lines (Kinghorn et al., 1999).

Xanthium strumarium L. fruits are used in traditional Chinese medicine for the treatment of sinusitis, rheumatism and skin pruritus; from this source a novel thiazinedione derivative has been reported (Ma et al., 1998). On the other hand, ethanolic extracts prepared from leaves of the same specie, showed trypanocidal activity in vitro; although in vivo trials, the extract proved to be toxic (Talakal et al., 1995). *Xanthium spinosum* L., known as “cepa caballo”, “abrojo chico” in Argentina or “clonqui” in Chile is used in ethnomedicine to treat digestive disorders due to its reputed anti-inflammatory, choleretic and anti-espasmodic properties (Alonso, 1998). In Argentina, some commercial herbal medicines contain aerial parts of this plant mixed with other species such as *Baccharis articulata* (Lam.) Pers., *Baccharis crispa* Sprengel, *Mentha piperita* L., and *Equisetum giganteum* L. Infusions of this herbs blend are commonly used for their digestive properties. The sesquiterpene pattern previously reported for

* Corresponding author. Fax: +54 2652 426711.
E-mail address: ctonn@unsl.edu.ar (C.E. Tonn).

0378-8741/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2005.02.042

Table 1
Gastroprotective effect of a single oral dose of compounds **1**–**10** (100 mg/kg) and omeprazole (60 mg/kg) on EtOH-induced lesions in rats[a]

Compound	Structure	Ulcer index	Inhibition (%)	Compound	Structure	Ulcer index	Inhibition (%)
1		0.12 ± 0.06*	97	**6**		1.00 ± 0.28*	79
2		0.87 ± 0.12*	82	**7**		4.66 ± 0.16	2
3		0.62 ± 0.37*	87	**8**		0.33 ± 0.16*	93
4		1.33 ± 0.16	74	**9**		3.66 ± 0.66	23
5		0.40 ± 0.18	92	**10**	 R= m-chlorobenzoyl	4.80 ± 0.12	0
Omeprazole	–	3.00 ± 0.28*	37	CMC + EtOH	–	4.75 ± 0.11	–

All values were expressed as mean ± S.E.M. Statistical analysis was carried out by an unpaired Student's *t*-test.
[a] CMC (carboxymethylcellulose) + EtOH served as the control.
* $p < 0.001$ significant differences from the control.

Xanthium spinosum (Omar et al., 1984; Abdei-Mogib et al., 1991) is close to that found by us for *Xanthium cavanillesii* Schouw [= *Xanthium strumarium* var. *canadense* (Mill.) Torr and Gray], and here reported.

Hemostatic, vermifuge, anti-inflammatory and detoxication properties of plant containing xanthanolides, and other sesquiterpenes, i.e. from *Carpesium longifolium* (Asteraceae), has been reported (Yang et al., 2003). Taking into account the central role that plays the γ-butirolactone moiety of some bioactive sesquiterpene lactones, the asymmetric synthesis of this functionality has been recently reported (Nosse et al., 2003).

A number of factors such as stress, chemical agents (ethanol, tobacco), bile salts, hyperosmolar NaCl, drugs (non-steroidal anti-inflammatory agents), may lead the gastroduodenal ulcer causing damage of the mucosa by a complex biologic process. The ulcer in the stomach or the duodenum seems to be an enigmatic interaction of several local changes and central nervous factors (Yetkin et al., 2004).

In an extensive research on the development of new anti-ulcer molecules from plant products, we have found that the guaianolide dehydroleucodine, isolated from *Artemisia douglasiana* Besser (Asteraceae), shows a cytoprotective effect and significantly prevents the gastric ulceration induced by various necrotizing agents (Giordano et al., 1992; María et al., 1998). From these studies it has been demonstrated that the α-methylene-γ-butyrolactone ring, presented in a series of sesquiterpene lactones and synthetic related compounds having a common Michael acceptor, plays a key role in the bioactivity (María et al., 2000).

Taking into account that the xanthanolide sesquiterpenes possess this kind of bioactive functionality, we engaged the isolation of sesquiterpenes from *Xanthium cavanillesii* Schouw. A previous phytochemical study of this species collected in Catamarca (Argentina) indicated that five seco-ambrosanolides (xanthanolides) are present in this plant (Riscala et al., 1994). The present study, which was developed using a plant samples collected in other phytogeographical region, reveals some differences in the secondary metabolites pattern.

In an attempt to establish the structural requirements for the gastric cytoprotective activity of the isolated xanthanolides and chemical derivatives (Table 1), a structure-activity study was carried out.

2. Materials and methods

2.1. General procedures

The ^{1}H NMR spectra were recorded at 200.13 MHz on a Bruker AC 200 instrument with TMS as internal standard. The ^{13}C NMR spectra were obtained with the same instruments at 50.23 MHz. Two-dimensional experiments were obtained using standard Bruker programs. IR spectra were recorded on an FT-IR Nicolet Protégé 460 spectrometer. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. All column chromatography were performed on Si gel G 70-230 mesh and Kieselgel 60 H using mixtures of *n*-hexane:EtOAc in increasing polarity as eluent. TLC was carried out on Si gel 60 F_{254} (0.2 mm-thick plates) using C_6H_6:1,4-dioxane:AcOH (30:5:1) as solvent. The terpene-containing fractions were detected by AcOH:H_2SO_4:H_2O (4:20:1) spray reagent followed by 150 °C oven heating.

2.2. Plant material

Xanthium cavanillesii Schouw was collected in El Volcan, Dpto. La Capital, San Luis, Argentina, in March 2002, and was identified by Prof. Luis A. Del Vitto. A herbarium sample is available from the Herbario of the Universidad Nacional de San Luis (voucher 8985-UNSL).

2.3. Extraction and isolation

The air-dried aerial parts of this plant (4.8 kg) were extracted with Me_2CO (three times) at room temperature for 5 days. The extract was concentrated in vacuum and the resultant dark brown syrup (560 g) was dissolved in a mixture of MeOH:H_2O (9:1), filtered, and extracted with *n*-hexane in order to remove pigment and fatty materials. The hydroalcoholic solution was diluted with H_2O (7:3) and then extracted with $CHCl_3$. After evaporation of the solvents the resulting residue was fractionated by repeated flash chromatography, and column chromatography. These purification techniques yielded xanthatin compounds **1** (3.8 g) (Omar et al., 1984; Marco et al., 1993), **2** (1.9 g) (Omar et al., 1984; Malik et al., 1993; Marco et al., 1993), **3** (950 mg) (Bohlmann et al., 1978; Marco et al., 1993), **4** (320 mg) (Marco et al., 1993), and **5** (253 mg) (Bohlmann et al., 1981). All spectral and physical data were in agreement with those previously reported.

2.4. Chemical derivatives

2.4.1. Compound *6*

Compound **1** (200 mg, 0.81 mmol) was mixed with 65.7 mg (1.21 mmol) of NaOMe in MeOH (2 ml) and left for 2 h at room temperature under stirring. After this time the mixture was neutralized using diluted HCl, and 5 ml of water were added to the crude. The hydroalcoholic layer was extracted twice with Et_2O and the organic phase was washed with aqueous Na_2CO_3, dried over anh. Na_2SO_4 and concentrated under vacuum. After purification by column chromatography, some 140 mg of the derivative **6** (as C-11 epimeric mixture) was recovered.

Compound **6**. Colourless oil; IR ν_{max}^{KBr}: 2933, 1778 (γ-lactone C=O), 1670 (ketone C=O), 1459, 1359, 1257, 1180, 1095, 981 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.05 (1H, d, J = 15.0, H-2), 6.31 (1H, dd, J = 8.6, 3.0 Hz, H-5), 6.20 (1H, d, J = 15.0, H-3), 4.28 (1H, ddd, J = 10.0, 10.0, 3.0 Hz, H-8), 3.71 (2H, m, H-13), 3.39 (3H, s, OMe), 3.05 (1H, m,

H-10), 2.81 (1H, m, H-6a), 2.55 (1H, m, H-11), 2.35 (2H, t, J = 1.5 Hz, H-9), 2.32 (3H, s, H-15), 2.12 (2H, m, H-6b and H-7), 1.20 (3H, d, J = 6.0 Hz, H-14); ^{13}C NMR ($CDCl_3$) δ 18.5 (C-14), 27.7 (C-15), 28.4 (C-6), 28.9 (C-10), 36.1 (C-9), 46.1 (C-7), 47.4 (C-11), 59.2 (O-Me), 69.1 (C-13), 81.4 (C-8), 124.4 (C-5), 139.2 (C-3), 144.4 (C-1), 148.5 (C-2), 175.4 (C-12), 198.5 (C-4).

2.4.2. Compound **7**

This derivative was prepared by reduction of the xanthanolide **2**. Some 400 mg (1.28 mmol) of compound **2** were dissolved in MeOH (5 ml) and 32 mg (0.84 mmol) of $NaBH_4$ were added. After stirring for 5 h the reaction was quenched using aqueous diluted HCl, concentrated under vacuum and diluted with water. The crude was extracted twice with Et_2O and the organic layer was washed with water, and dried with anh. Na_2SO_4. After evaporation the residue was purified by column chromatography. Some 310 mg of compound **7** were obtained. Compound **7**. Colorless oil. IR ν_{max}^{KBr}: 1770 (C=O), 1450, 1378, 1224, 1186, 979.6, 852.4 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 5.85 (1H, dd, J = 8.6, 3 Hz, H-5), 4.31 (3H, m, H-2, H-4 and H-8), 4.13 (1H, m, H-4), 2.65 (1H, m, H-10), 2.32 (2H, m, H-7 and H-9b), 2.08 (2H, m, H-6), 1.68 (2H, m, H-3), 1.60 (2H, m, H-11 and H-9a), 1.28 (3H, d, J = 6.0 Hz, H-14), 1.25 (3H, d, J = 6.0 Hz, H-13), 1.20 (3H, d, J = 6.0 Hz, H-15); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 12.4 (C-14), 19.1 (C-15), 23.4 (C-13), 26.2 (C-6), 29.9 (C-10), 36.2 (C-9), 41.5 (C-7), 42.5 (C-3), 51.5 (C-11), 65.5 (C-4), 75.5 (C-2), 82.2 (C-8), 123.2 (C-5), 150 (C-1), 178.6 (C-12).

2.4.3. Compound **8**

To 200 mg (0.81 mmol) of compound **1** dissolved in 10 ml of $CHCl_3$, *m*-chloroperbenzoic acid (181.1 mg, 1.05 mmol) and $NaHCO_3$ (50 mg, 0.59 mmol), were added. After stirring for 24 h, the usual work and column chromatography purification afforded 120 mg of the α-epoxide derivative (**8**) and 56 mg of the β-diastereomer. All spectral and physical data were identical with those previously reported (Omar et al., 1984; Marco et al., 1993).

2.4.4. Compound **9**

To a stirring solution of compound **2** (200 mg, 0.64 mmol) in 20 ml of CH_2Cl_2 under argon at room temperature, piperidine was added (66 mg, 0.76 mol). After 12 h the solvent was evaporated under vacuum and the residue was subjected to column chromatography purification. Some 180 mg of compound **9** were recovered as a C-11 epimeric mixture.

Compound **9**. Colorless oil. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 5.78 (1H, dd, J = 9.0, 3.0 Hz, H-5), 5.18 (1H, dd, J = 7.0, 7.0 Hz, H-2), 4.30 (1H, ddd, J = 12.0, 10.0, 3.0 Hz, H-8), 3.95 (1H, ddq, J = 8.0, 4.0, 6.0 Hz, H-4), 2.78 (1H, m, H-10), 2.79 (1H, m, H-13b), 2.44 (1H, m, H-13a), 2.11 (3H, s, OAc), 1.45–1.80 (10 H, m, CH_2 piperidin moiety), 1.28 (3H, d, J = 6.0 Hz, H-15), 1.23 (3H, d, J = 7 Hz, H-14); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 19.2 (C-14), 20.6 (C-15), 21.2 (OAc), 29.8 (C-10), 29.4 (C-6), 36.4 (C-9), 42.6 (C-3), 44.1 (C-11), 58.3 (C-13), 68.3 (C-4), 74.2 (C-2), 82.1 (C-8), 124.1 (C-5), 148.8 (C-1), 171.4 (C-12), 177.6 (OAc). Five methylene carbons at 54.7 (2C), 25.8, 24.1 (2C) (piperidin moiety).

2.4.5. Compound ***10***

This compound was obtained as a side-product during the previously described epoxidation reaction (see compound **8** preparation). This derivative was the result of the oxirane ring opening due to a nucleophilic attack of the *m*-chlorobenzoate ion on the α-epoxide (**8**). Compound **10**. Colorless oil. IR ν_{max}^{KBr}: 3450 (OH), 2950, 2800, 1750 (C=O), 1780 (γ-lactone C=O), 1410, 1290, 1150 (C–OH), 750 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.40–7.90 (m, four aromatic protons), 7.71 (1H, d, J = 9.0 Hz, H-2), 6.22 (1H, d, J = 1.0 Hz, H-13b), 5.71 (1H, d, J = 9.0 Hz, H-3), 5.59 (1H, d, J = 1.0 Hz, H-13a), 5.32 (1H, t, J = 1.0, 0.5 Hz, H-5), 4.01 (1H, ddd, J = 10.0, 10.0, 3.0 Hz H-8), 3.23 (2H, m, H-7 and H-10), 2.49 (1H, dt, J = 13.5, 1.0, 0.5 Hz, H-6b), 2.19 (3H, s, H-15), 2.03 (2H, m, H-9), 1.53 (1H, m, H-6a), 1.25 (3H, d, J = 6.0 Hz, H-14); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 20.0 (C-14), 20.8 (C-15), 26.1 (C-10), 37.9 (C-6), 39.1 (C-7 and C-9), 66.6 (C-5), 82.5 (C-8), 86.8 (C-2), 119.1 (C-3), 120.9 (C-13), 139.3 (C-11), 152.2 (C-1), 168.7 (C-4), 170.3 (C-12) [127.9, 129.8 (two carbons), 130.5, 133.7, 134.6, 163.4; *m*-chlorobenzoyl moiety].

2.5. Biological experiments

2.5.1. Animals

Male Wistar rats, weighing 200–250 g were employed. Animals were provided with standard food and water ad libitum and were maintained at a constant temperature (22 ± 1 °C) and humidity of 55 ± 5%. The animals were randomly assigned to different groups (n = 5–8), and a period of 4 days was allowed for adaptation before each experiment. All experiments were in compliance with the ANMAT no. 7344/96 for animals care guideline. The animals were deprived of food for 24 h prior to starting the experiments and had free access to water.

2.5.2. Induction of gastric lesions

Gastric lesions were produced according to the method of Robert et al. (1979). Wistar rats were fasted for 24 h and 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 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 from 0 (no erosions) to 5 (maximal damage) (Giordano et al., 1992). The results were expressed in terms of an ulcer index, which is the average severity of erosions per rat for each group. Compounds were prepared just before the experiment (100 mg/kg), suspended in 0.4% carboxymethyl cellulose (CMC) and were given 60 min prior

Table 2
Effect of xanthatin (Xant.) (compound **1**) on gastric ulcers induced by different necrotizing agents (0.6N HCl and 0.2N NaOH) in Wistar rats

Treatment	Xanthatin dose (oral, mg/kg body weight)	Ulcer index	Percentage inhibition of ulceration
HCl (control)	0	4.50 ± 0.26	–
HCl + Xant.	6.25	3.75 ± 0.25	17
HCl + Xant.	12.50	1.87 ± 0.23**	58
HCl + Xant.	25.00	0.60 ± 0.10**	96
HCl + Xant.	50.00	0.50 ± 0.20**	97
HCl + Xant.	100.00	0.87 ± 0.37**	94
NaOH (control)	0	4.50 ± 0.22	–
NaOH + Xant.	6.25	3.83 ± 0.44	15
NaOH + Xant.	12.50	1.87 ± 1.04*	58
NaOH + Xant.	25.00	0.40 ± 0.18**	91
NaOH + Xant.	50.00	0.30 ± 0.12**	93
NaOH + Xant.	100.00	0.16 ± 0.16**	96

All values were expressed as mean ± S.E.M.
* $p < 0.01$ significant differences from the control.
** $p < 0.001$ significant differences from the control.

to the necrotizing agent. Omeprazole was administered p.o. at a dose of 60 mg/kg. The control rats were given CMC and 1 ml absolute ethanol (p.o.).

2.5.3. Gastric ulcers induced by necrotizing agents

Xanthatin was investigated for its ability to protect the gastric mucosa against injuries caused by necrotizing agents. Gastric lesions were produced according to the method of Robert et al. (1979). Some 1 ml of necrotizing agent (0.6N HCl or 0.2N NaOH) was administered intragastrically to rats that had been fasted for 24 h with access to water ad libitum. Xanthatin was given intragastrically 60 min before the necrotizing agents. The animals were killed 1 h after the administration of necrotizing agents, and examined for stomach ulcers. Results are shown in Table 2.

2.6. Statistics

Results are given as mean ± S.E.M. The statistical significance of the data was evaluated by Student's *t*-test for unpaired data and *p* values lower than 0.05 were considered significant.

2.7. Computational methods

Conformational search was made by the Montecarlo method, using the Merck Molecular Force Field (MMFF94), which appears to be quite well suited for this purpose, except when the anomeric effect may lead to significant conformational preferences. The Spartan'04® (PC Spartan, 1999; Pungitore et al., 2004) routine allows varying simultaneously several dihedral angles even those corresponding to flexible rings. In the case of a ring, "rotation" means that the atom is to be "puckered-up" and "puckered-down" (restricted rotation). Fig. 1 shows, surrounded by a circle, the dihedral angles that were varied for compound **1**. The energy of each conformer was calculated using the AM1 semi-empirical method. Results are shown in Table 3.

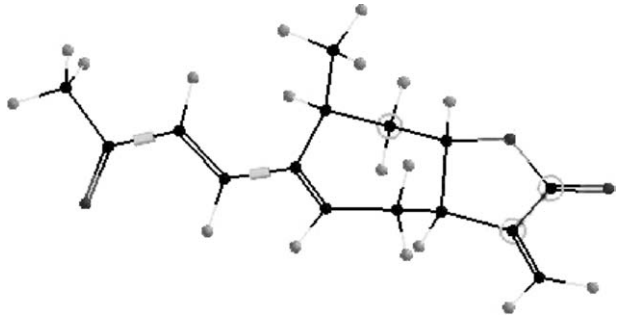


Fig. 1. The circles indicates the angles that were varied in the conformational study of compound **1**.

Table 3
Energy differences between the two lowest energy comformers for some natural xanthanolides and chemical derivatives

Compound	ΔΔHf (kcal/mol)
1	0.4925
2	0.2019
3	0.6550
5	0.0255
8	0.9712
10	1.9700

3. Results and discussion

Compounds **1**–**3** (Table 1) showed an important cytoprotective activity against ulcer induced by absolute ethanol. The observed values for both ulcer index, and percentage of ulcer inhibition were comparable to those previously reported for dehydroleucodine (DhL) the most active guaianolide-type sesquiterpene lactone possessing this kind of bioactivity (Giordano et al., 1992). It has been reported that the gastric cytoprotection might be mediated by at least two different mechanisms, one of them through prostaglandin synthesis, and the second one by increasing the mucosal glycoproteins production (Guardia et al., 1994). On the other hand, the cytoprotective activity of sesquiterpene lactones has been attributed to the presence of a non-hindered electrophilic acceptor in

the molecular structure that could interact with sulfhydryl-containing compounds of the gastric mucosa, namely reduced glutathione and others (María et al., 2000).

Compound **1**, the most abundant xanthanolide isolated from *Xanthium cavanillesii*, shows and exocyclic methylene group at C-13 conjugated with the lactone carbonyl group (C-12) as well as a second potential reactive site at C-5 as part of an α,β,γ,δ-unsaturated methyl-ketone substructure. Both C-13 and C-5 (this in minor extension) constitutes electrophilic centers capable of forming Michael adducts by the addition of nucleophiles as the aforementioned sulfhydryl groups from the biological molecules presented in the gastric mucosa.

Although compounds **2** and **3** only exhibited the C-13 carbon as a Michael addition reactive position, from the observed cytoprotection results the α-methylene-γ-butyrolactone is confirmed as the key functional group responsible of the bioactivity (María et al., 2000).

Previously it has been informed that some α,β-unsaturated carboxylic acids present a low cytoprotective capacity compared to α-exomethylen-γ-butyrolactones (María et al., 2000). From the results obtained when compound **4** was assayed, this behavior seemed also to be evidenced in xanthanolides. The high ulcer inhibition value showed by compound **5** was in agreement with this general observation.

Compound **6** was prepared from the xanthanolide **1** by a methoxyde ion attack on C-13 throughout a Michael addition. This chemical transformation was carried out in order to blocking one reactive center (C-13), and therefore evaluates the α,β,γ,δ-unsaturated methyl-ketone moiety role in the bioactivity expression. When compound **6** was assayed a significant cytoprotection (79%) was still observed. From this results it is possible to conclude that this Michael acceptor could act as a second structural requirement to draw out the here investigated bioactivity.

Results obtained using compound **7**, which not exhibited any theoretically reactive center, seem to confirm this hypothesis. Additionally the C-1–C-5-α-oxirane derivative **8**, where both C-2 and C-13 carbons constitutes suitable Michael acceptors, showed a strong activity.

Definitive proofs about the cooperative behavior of the two reactive sites were recovered from the biological evaluation of compound **9**. In this case the loss of bioactivity was dramatic as was described for compound **7**. Noteworthy that the derivative **9** has blocked the C-13 position and the side chain does not possess a conjugated carbonyl group.

The inhibitory action exerted by xanthatin (compound **1**) on the ulcers induced by 0.6N HCl and 0.2N NaOH was highly significant, the inhibition of ulceration being in the range of 58–96% (Table 2).

The *m*-chlorobenzoylester **10**, whose stereochemistry was extensively studied using 2D NMR experiments (NOESY), was clearly inactive. This compound exhibited two potentially active places; they are the α,β-unsaturated methylketone group, and the unsaturated lactone moiety. The C-1 hydroxyl group now protects the C-2 position from a nucleophilic attack. It has been reported that the presence of this kind of functionality is in agreement with the loss of cytoprotective activity (Giordano et al., 1992). Noteworthy, the ^{13}C NMR spectral data show that the C-2 position in compound **1** ($\delta = 149.5$ ppm) was more deshielded that the corresponding to compound **10** ($\delta = 86.8$ ppm); the chemical shift of this carbon could be correlated with its electrophyle properties.

On the other hand, although the α,β-unsaturated-γ-lactone moiety is present, would seem that the *m*-chlorobenzoil group at C-5 would introduce some stereo-electronic factors that do not allow its expression as Michael acceptor. These discrepancies may have been caused by different conformational flexibility. In this sense it has been reported that the increase of the molecular flexibility enhance the possibility of reacting with sulfhydryl groups (Beekman et al., 1997).

In order to explore this possibility, a theoretical conformational analysis was performed with a Montecarlo method using the Merck Molecular Force Field (MMFF94) includes in the Spartan'04® software (PC Spartan, 1999; Pungitore et al., 2004).

Compounds selected for this study were molecules where the α,β-unsaturated-γ-lactone moiety is present without any interference such as compounds **1**, **2**, **3**, **5**, **8** (all bioactives), and **10** (no bioactive). All of these compounds showed conformations of "chair", "boat" or "twisted boat" type for the 7-membered ring (Fig. 2). The lowest energy conformers of each type thus obtained, was minimized using a semi-empirical method (AM1). Energy differences among the two lowest energy conformer are shown in Table 3.

The energy difference between the two lowest energy conformers for compound **10** was significantly greater than the difference for the other compounds. This could suggest that conformational interchanging is more difficult and, therefore, this molecule shows less flexibility. This agrees with the already suggested hypothesis (Beekman et al., 1997).

In conclusion, the proposed mechanism would involve a nucleophylic attack of the sulfhydryl group to the β carbon of the Michael acceptors of the assayed compounds. This seems to confirm the previously reported structural requirement for sesquiterpene lactones acting as anti-ulcerogenic agents (Giordano et al., 1992; María et al., 2000).

Recently, the anti-ulcerogenic bioactivity elicited by sesquiterpene lactones from *Centaura solstitialis* L. ssp. *solstitialis* has been reported (Yesilada et al., 2004). In this case three active compounds possessing guaianolide skeleton were isolated, and alone one of them (chlorojancrin) showed the α-methylene-γ-butyrolactone moiety, to our understand the main responsible functionality for the bioactivity. Although this structural requirement was absent in the other two active compounds, an enzymatic oxidation of the C-3 hydroxyl group could drive to an exocyclic α,β-unsaturated carbonyl group on the five membered ring, clearly suitable as Michael acceptor (María et al., 2000). The facile oxidation of the allylic hydroxyl group could be supported by thermodynamic considerations.

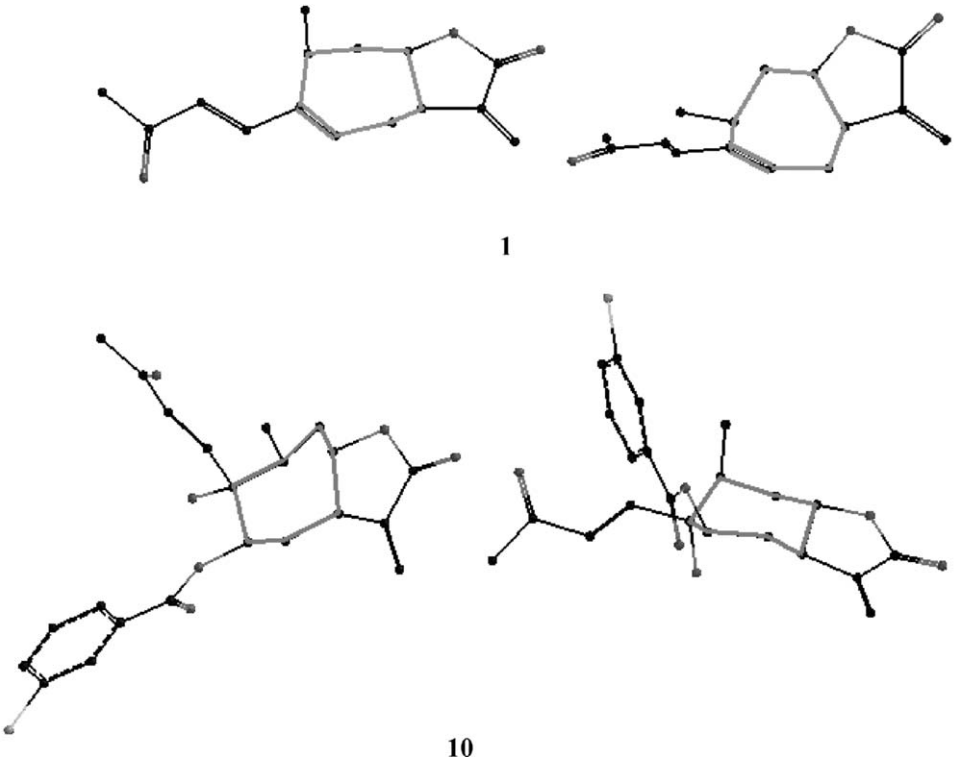


Fig. 2. Lowest energy conformers for compounds **1** and **10**.

However, caution is required in such interpretation and additional analysis will be necessary to confirm this speculation.

Acknowledgements

Financial support from CONICET (PIP 02431), ANPCyT (PICT 2002/10714), and UNSL (Projects 22/Q205; 8504), is gratefully acknowledged. We thank Prof. L.A. del Vitto (Herbario UNSL) for plant identification. This work is a part of the Doctoral thesis of Laura S. Favier.

References

Abdei-Mogib, M., Dawidar, A.M., Metwally, M.A., Abou-Elzahab, M., 1991. Xanthanolides from *Xanthium spinosum*. Phytochemistry 30, 3461–3462.

Alonso, J.R., 1998. Tratado de Fitomedicina. ISIS Ediciones, Argentina, p. 386.

Beekman, A.C., Woerdenbag, H.J., van Uden, W., Pras, N., Konings, A.W.T., Wikstrom, H.V., Schmidt, T.J., 1997. Structure-cytotoxicity relationships of some helenanolide-type sesquiterpene lactones. Journal of Natural Products 60, 252–257.

Bohlmann, F., Jakupovic, J., Schuster, A., 1981. Further eudesmanolides and xanthanolides from *Telekia speciosa*. Phytochemistry 20, 1891–1893.

Bohlmann, F., Mahanta, P.K., Jakupovic, J., Rastogi, R.C., Natu, A.A., 1978. New sesquiterpene lactones from *Inula* species. Phytochemistry 17, 1165–1172.

Giordano, O.S., Pestchanker, M.J., Guerreiro, E., Saad, J.R., Enriz, R.D., Rodríguez, A.M., Jáuregui, E., Guzmán, J., María, A.O., Wendel, G.H., 1992. Structure-activity relationship in the gastric cytoprotective effect of several sesquiterpene lactones. Journal of Medicinal Chemistry 35, 2452–2458.

Guardia, T., Guzmán, J., Pestchanker, M.J., Guerreiro, E., Giordano, O.S., 1994. Mucus synthesis and sulfhydryl groups in cytoprotection mediated by dehydroleucodine, a sesquiterpene lactone. Journal of Natural Products 57, 507–509.

Kinghorn, A.D., Farnsworth, N.R., Soejarto, D.D., Cordell, G.A., Pezutto, J.M., Udeani, G.O., Wani, M.C., Wall, M.E., Navarro, H.A., Kramer, R.A., Menendez, A.T., Fairchild, C.R., Lane, K.E., Forenza, S., Vyas, D.M., Lam, K.S., Shu, Y.Z., 1999. Novel strategies for the discovery of plant-derived anti-cancer agents. Pure and Applied Chemistry 71, 1611–1618.

Ma, Y.T., Huang, M.C., Hsu, F.L., Chang, H.F., 1998. Thiazinedione from *Xanthium strumarium*. Phytochemistry 48, 1083–1085.

Malik, M.S., Sangwan, N.K., Dhindsa, K.S., 1993. Xanthanolides from *Xanthium strumarium*. Phytochemistry 32, 206–207.

Marco, J.A., Sanz-Cervera, A.F., Corral, J., Carda, M., Jakupovic, J., 1993. Xanthanolides from *Xanthium*: absolute configuration of xanthanol, isoxanthanol and their C-4 epimers. Phytochemistry 34, 1569–1576.

María, A.O.M., Donadel, O., Wendel, G.H., Guzmán, J.A., Guerreiro, E., Giordano, O.S., 2000. Gastric anti-ulcer activity of several α,β-unsaturated carbonyl compounds in rats. Biological and Pharmaceutical Bulletin 23, 555–557.

María, A.O.M., Franchi, A.M., Wendel, G.H., Gimeno, M., Guzmán, J.A., Giordano, O.S., Guerreiro, E., 1998. Gastric cytoprotective activity of dehydroleucodine in rats. Role of prostaglandins. Biological and Pharmaceutical Bulletin 21, 335–338.

Nosse, B., Chhor, R.B., Jeong, W.B., Bohm, C., Reiser, O., 2003. Facile asymmetric synthesis of the core nuclei of xanthanolides, guaianolides, and eudesmanolides. Organic Letters 5, 941–944.

Omar, A.A., Elrashidy, E.M., Ghazy, N.A., Metwally, A.M., Ziesche, J., Bohlmann, F., 1984. Xanthanolides from *Xanthium spinosum*. Phytochemistry 23, 915–916.

PC Spartan Pro Ver. 1. Wavefunction, Inc., Irvine, CA: 1999. Reg. #Ppro1-002353.

Pungitore, C.R., Juri Ayub, M., García, M., Borkowski, E., Sosa, M.E., Ciuffo, G., Giordano, O.S., Tonn, C.E., 2004. Iridoids as allelochemicals and DNA Polymerase inhibitors. Journal of Natural Products 67, 357–361.

Riscala, E.C., de Fortuna, M.A., Catalán, C.A.N., Díaz, J.G., Herz, W., 1994. Xanthanolides and bis-norxanthanolides from *Xanthium cavanillesii*. Phytochemistry 35, 1588–1589.

Robert, A., Nezamis, J.E., Lancaster, C., Hanchar, A.J., 1979. Cytoprotection by prostaglandins in rats. Gastroenterology 77, 433–443.

Sato, Y., Oketani, H., Yamada, T., Singyouchi, K., Ohtsubo, T., Kihara, M., Shibata, H., Hi, T., 1997. A xanthanolide with potent anti-bacterial activity against methicillin-resistant *Staphylococcus aureus*. The Journal of Pharmacy and Pharmacology 49, 1042–1044.

Saxena, V.K., Mondal, S.K., 1994. A xanthanolide from *Xanthium strumarium*. Phytochemistry 35, 1080–1082.

Talakal, T.S., Dwivedi, S.K., Sharma, S.R., 1995. In vitro and in vivo anti-trypanosomal activity of *Xanthium strumarium* leaves. Journal of Ethnopharmacology 49, 141–145.

Yang, C., Yuan, C., Jia, Z., 2003. Xanthanolides, germacranolides and other constituents from *Carpesium longifolium*. Journal of Natural Products 66, 1554–1557.

Yesilada, E., Gürbüz, I., Bedir, E., Tatli, I., Khan, I.A., 2004. Isolation of anti-ulcerogenic sesquiterpenes lactones from *Centaurea solstitialis* L. spp. *solstitialis* through bioassay-guide fractionation procedures in rats. Journal of Ethnopharmacology 95, 213–219.

Yetkin, G., Çelebi, N., Özer, Ç., Gönül, B., Özoğul, C., 2004. The healing effect of TGF-α on gastric ulcer induced by acetylsalicylic acid in rats. International Journal of Pharmaceutics 277, 163–172.