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Gastroprotective activity and cytotoxic effect of cyperenoic acid derivatives

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Abstract

The gastroprotective effect of the sesquiterpene cyperenoic acid and seven semi-synthetic derivatives was assessed in the HCl/ethanol-induced gastric ulcer model in mice. At doses of 25, 50 and 100 mg kg^{-1}, cyperenoic acid showed a dose-dependent gastroprotective effect reducing the lesions by 45 and 75% at 50 and 100 mg kg^{-1}, respectively. Seven derivatives of the sesquiterpene were prepared and their gastroprotective activity compared at 50 mg kg^{-1}. The cytotoxicity of the compounds was evaluated in fibroblasts and AGS cells. At 50 mg kg^{-1}, patchoulan-15-oic acid (compound **8**) presented the best gastroprotective effect, reducing the gastric lesions by 86%, with a similar effect to lansoprazole at 20 mg kg^{-1}. The gastroprotective effect of cyperenol, cyperenoic acid methyl ester and the ethylamide and butylamide from cyperenoic acid were in the same range, reducing the gastric lesions by 72–77%. Cyperenol and cyperenoic acid methyl ester, however, were more cytotoxic with IC50 (concentration that produces a 50% inhibitory effect) values of 44 and 75, 48 and 75 μM against AGS cells and fibroblasts, respectively. The best gastroprotective effect with lower cytotoxicity was found for the compound **8**, cyperenoic acid and the *p*-anisidyl derivative **7**.

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Introduction

An infusion or decoction of *Jatropha isabelli* Muell. Arg. (Euphorbiaceae) rhizomes is used in Paraguayan traditional medicine as a digestive and an abortifacient and to treat rheumatism and human gout. From the rhizomes of this crude drug, known in Paraguay as yaguá rová, the sesquiterpene cyperenoic acid and the diterpene jatrophone were isolated and identified by spectroscopic means as the main terpenoid constituents (Schmeda-Hirschmann et al 1996). It has been shown that some terpenoids, including diterpenes, triterpenes and sesquiterpenes, present gastroprotective activity in different models of induced gastric lesions in animals (Schmeda-Hirschmann & Yesilada 2005; Schmeda-Hirschmann et al 2005a, b; Sepúlveda et al 2005). Recently, the in-vitro cytoprotective effect of some terpenoids on human gastric cell cultures has been also reported (Rodríguez et al 2005, 2006).

Most studies on the gastroprotective effect of sesquiterpenes have been focused on the sesquiterpene lactones (Giordano et al 1990; Lewis & Hanson 1991; Foglio et al 2002), 11-hydroxy-4-amorphen-15-oic acid derivatives (Reyes et al 2005) and polygodial and its derivatives (Matsuda et al 2002). Little is known about the mechanisms involved in the gastroprotective effect of plant sesquiterpenes. Recently, Matsuda et al (2002) and Pongpiriyadacha et al (2003) have reported that the mode of action underlying the gastroprotective activity of polygodial and related compounds may include effects on endogenous prostaglandins, sulfhydryl compounds, nitric oxide and vanilloid receptors. Foglio et al (2002) have suggested that the sesquiterpene lactones dihydroepideoxyarteannuin B and deoxyartemisinin act as gastroprotective compounds, increasing the prostaglandin content.

Following our studies on the gastroprotective effect of naturally occurring and modified terpenoids from South American crude drugs, we now report the gastroprotective effect of cyperenoic acid and some semi-synthetic derivatives, in the HCl/EtOH-induced gastric ulcer model in mice as well as the cytotoxicity towards human lung fibroblasts and AGS cells.

Materials and Methods

Compounds

Melting points were determined on a Koffler hot stage apparatus (Electrothermal 9100) and were uncorrected. Optical rotations were obtained for solutions in $CHCl_3$ (concentrations expressed in g/100 mL) on a Jasco DIP 370 polarimeter. IR spectra were recorded on a Nicolet Nexus FT-IR instrument. ^{1}H NMR spectra were recorded at 400 MHz and ^{13}C NMR data were obtained at 100 MHz on a Bruker spectrometer (δ scale). TLC spots were visualized by spraying the chromatograms with *p*-anisaldehyde–ethanol–acetic acid–H_2SO_4 (2:170:20:10 v/v) and heating at 110°C for 3 min. Column chromatography was performed over Merck Kieselgel 60, particle size 0.063–0.200 mm. Mass spectra are presented as m/z (% rel. int.). THF was distilled from sodium and pyridine and dichloromethane were distilled from calcium hydride under N_2 atmosphere. All reactions were carried out under an inert dry nitrogen atmosphere. Benzene was distilled from calcium chloride and kept over sodium.

Isolation of cyperenoic acid

Rhizomes of the crude drug yaguá rová were collected at Altos, Departamento Cordillera, Paraguay, in May 2004. The plant belongs to the same population surveyed in 1994 and was documented with the voucher herbarium specimen Schmeda 1594, deposited at the Smithsonian Institution, Washington DC, USA. The rhizomes were sliced, dried at room temperature and powdered. The air-dried, powdered rhizomes of *Jatropha isabelli* (3.0 kg) were successively extracted under reflux (3 times, 5 L each time, 30 min/extraction) with petroleum ether (PE), ethyl acetate (EtOAc) and methanol (MeOH). After concentration under reduced pressure and lyophilization, the w/w yields from the dry starting material was as follows: PE 50.5 g (1.68%), EtOAc 68.3 g (2.27%) and MeOH 185 g (6.16%). Cyperenoic acid was obtained from the PE and EtOAc extracts after column chromatography on silica gel (2.0 kg, particle size: 200–500 μm, column length 65 cm, i.d. 9 cm). Some 31 fractions of 2.0 L each were obtained and pooled together according to the TLC patterns. Cyperenoic acid was found in the fractions eluted with PE–EtOAc 8:2 and 7:3. After recrystallization, some 2.6 g of the sesquiterpene was obtained (w/w yield: 0.087%).

Synthesis of cyperenoic acid derivatives

Cyperenoic acid **3** was methylated with CH_2N_2 to afford the methyl ester **4** (97%). Reduction of **4** with $LiAlH_4$ in THF yielded the alcohol **1** (37%). Compound **2** (78%) was obtained by acetylation of the alcohol **1**. Compounds **5, 6** and **7** were prepared by treating **3** in dry benzene $SOCl_2$/TEA with different amines. The following amines were used to prepare the corresponding amides: ethylamine (compound **5**), butylamine (compound **6**) and *p*-anisidine (compound **7**) with yields of 28%, 79% and 12%, respectively. Compound **8** was prepared by reduction of the double bond of compound **3** with H_2/Pd/C. The structures of compounds **1**–**8** are shown in Figure 1.

Cyperenol (4-patchoulen-15-ol)
Compound **1**: colourless crystals, mp 92–94°C. MS m/z (% rel. int.): 220.1765 (100) (calcd for $C_{15}H_{24}O$: 220.1827), 205 (17), 202 (26), 191 (19), 189 (20), 187 (39), 177 (9), 159 (46), 145 (27), 131 (22), 117 (14), 105 (21), 91 (24), 79 (12), 55 (12). FT-IR (KBr, cm^{-1}): 3383, 1710, 1692, 1259, 1055. $[\alpha]_D^{20}$: −13.8 (c = 0.29, $CHCl_3$).

Cyperenyl acetate
Compound **2**: pale yellow oil. MS m/z (% rel. int.): 262.1933 (27) (calcd for $C_{17}H_{26}O_2$: 262.1933), 220 (20), 219 (47), 218 (24), 205 (16), 204 (95), 203 (33), 189 (23), 187 (16), 159 (100), 147 (34), 145 (30), 133 (16), 131 (19), 105 (23), 91 (21). FT-IR (film, cm^{-1}): 2924, 1744, 1226, 1023. $[\alpha]_D^{20}$: –9.1 (c = 0.22, $CHCl_3$).

Cyperenoic acid (4-patchoulen-15-oic acid)
Compound **3**: colourless crystals, mp 162–164°C. MS m/z (% rel. int.): 234.1620 (calcd for $C_{15}H_{22}O_2$: 234.1620). FT-IR (KBr, cm^{-1}): 2955, 1676, 1649, 1434, 1287, 950. $[\alpha]_D^{20}$: −16.0 (c = 0.5, $CHCl_3$).

Cyperenoic acid methyl ester (methyl cyperenoate)
Compound **4**: pale yellow oil. MS m/z (% rel. int.): 248.1776 (100) (calcd for $C_{16}H_{24}O_2$: 248.1776), 233 (8), 217 (17), 216 (32), 205 (28), 192 (30), 191 (11), 189 (13), 177 (13), 145 (22), 133 (16), 117 (15), 91 (14). FT-IR (film, cm^{-1}): 2950, 1710, 1665, 1259, 1055. $[\alpha]_D^{20}$: −10.0 (c = 0.5, $CHCl_3$).

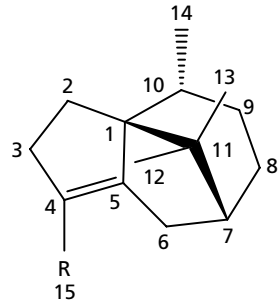
N-*Ethyl-cyperen-15-amide*
Compound **5**: colourless crystals, mp 124–126°C. MS m/z (% rel. int.): 261.2093 (100) (calcd for $C_{17}H_{27}NO$: 261.2093), 246 (17), 218 (47), 189 (11), 147(6), 133 (5), 119 (6), 117 (5), 105 (7), 91 (9). FT-IR (KBr, cm^{-1}): 3283, 2932,1671, 1621, 1532, 1285, 1145. $[\alpha]_D^{20}$: −5.0 (c = 0.2, $CHCl_3$).

N-*Butyl-cyperen-15-amide*
Compound **6**: brown resin. MS m/z (% rel. int.): 289.2406 (75) (calcd for $C_{19}H_{31}NO$: 289.2406), 274 (23), 256 (27), 254 (27), 246 (38), 232 (25), 223 (27), 217 (47), 216 (38), 213 (26), 189 (29), 133 (31), 127 (56), 91 (44), 81 (30), 72 (43), 69 (28), 64 (65), 57 (100), 55 (78). FT-IR (film, cm^{-1}): 3324, 2956, 1668, 1623, 1526, 1248. $[\alpha]_D^{20}$: −10,3 (c = 0.29, $CHCl_3$).

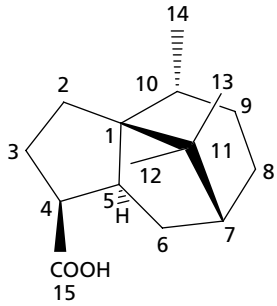
N-*(*p*-Anisidyl)-cyperen-15-amide*
Compound **7**: brown oil. MS m/z (% rel. int.): 339.2198 (100) (calcd for $C_{22}H_{29}NO_2$: 339.2198), 324.18 (6), 218 (16), 217 (94), 189 (9), 133 (7), 123 (9), 122 (5), 119 (5), 105 (7), 91 (8), 69 (4). FT-IR (KBr, cm^{-1}): 3310, 2925, 1665, 1630, 1512, 1246, 1036, 828. $[\alpha]_D^{20}$: −10.0 (c = 0.1, $CHCl_3$).

Patchoulan-15-oic acid
Compound **8**: colourless crystals, mp 90–93°C, elemental analysis: calcd.: C: 76.23; H: 10.24; found: C: 76.00; H: 10.15 ($C_{15}H_{24}O_2$), FT-IR (KBr, cm^{-1}): 2956, 1697, 1420, 1281, 950. $[\alpha]_D^{20}$: −53.6 (c = 0.28, $CHCl_3$). Obtained as a 2:1 mixture of **8a** and **8b**.

Compound	R
1	CH_2OH
2	CH_2OCOCH_3
3	COOH
4	$COOCH_3$
5	$CONHCH_2CH_3$
6	$CONHCH_2CH_2CH_2CH_3$
7	$CONHPhOCH_3$



Compound **8 a**



Compound **8 b**

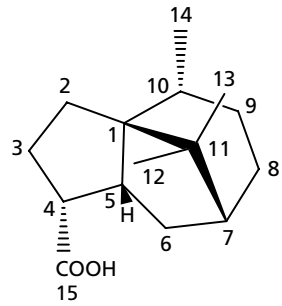


Figure 1 structures of compounds **1–8.**

Animals

Fasted Swiss albino mice, 30 ± 3 g, were used. Fasting (24 h) was used before ulcerogenic assays because the reference compound (lansoprazole), the sesquiterpenes and their derivatives were administered orally. The mice were fed on certified Champion diet with free access to water under standard conditions of 12-h dark–light, 50% relative humidity and 22°C room temperature. The protocols were approved by the Universidad de Talca Institutional Animal Care and Use Committee that follows the recommendations of the Canadian Council on Animal Care (Olfert et al 1993).

HCl/EtOH-induced lesions

The gastroprotective activity of the compounds was assessed in the HCl/EtOH-induced lesion model as described previously (Schmeda-Hirschmann et al 2005b). Mice were randomly distributed into groups of eight each and fasted for 24 h with free access to water before the experiment. For comparison of the cyperenoic acid derivatives, a single oral dose of 50 $mg\,kg^{-1}$ was selected because in a previous experiment we determined that the lesion index was reduced by about 50% by cyperenoic acid at 50 $mg\,kg^{-1}$. Fifty minutes after oral administration of the compounds, lansoprazole (2-[[[3-methyl-4-(2,2,2-trifluroethoxy)-2-pyridyl]methyl]sulfinyl] benzimidazole) (20 $mg\,kg^{-1}$) or 12% Tween 80 (10 $mL\,kg^{-1}$), all groups were orally treated with 0.2 mL of a solution containing 0.3 M HCl–60% EtOH (HCl/EtOH) for gastric lesion induction. Mice were sacrificed 1 h after the administration of the HCl/EtOH solution, and the stomachs were excised and inflated by injection of saline (1 mL). The ulcerated stomachs were fixed in 5% formalin for 30 min and opened along the greater curvature. Gastric damage visible to the naked eye was observed in the gastric mucosa as elongated black-red lines, parallel to the long axis of the stomach similar to the HCl/EtOH-induced lesions in rats. The length (mm) of each lesion was measured, and the lesion index was expressed as the sum of the length of all lesions.

MRC-5 cell culture

The cytotoxic effect of the assayed compounds, expressed as cell viability, was assessed on a permanent fibroblast cell line derived from human lung (MRC-5) (ATCC CCL-171). MRC-5 fibroblasts were grown as monolayers in minimum essential Eagle medium (MEM), with Earle's salts, 2 mM L-glutamine and 2.2 $g\,L^{-1}$ sodium bicarbonate, supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 $IU\,mL^{-1}$ penicillin and 100 $\mu g\,mL^{-1}$ streptomycin in a humidified incubator with 5% CO_2 in air at 37°C. Cell passage was maintained between 10 and 16. The medium was changed every 2 days.

AGS cell culture

The cytotoxic effect of the assayed compounds, expressed as cell viability, was assessed on a permanent human epithelial gastric cell line (AGS) (ATCC CRL-1739). The AGS cells were grown as monolayers in Ham F-12 medium containing 1 mM L-glutamine and 1.5 $g L^{-1}$ sodium bicarbonate, supplemented with 10% heat-inactivated FBS, 100 IU mL^{-1} penicillin and 100 $\mu g mL^{-1}$ streptomycin in a humidified incubator with 5% CO_2 in air at 37°C. The cell passage was maintained between 42 and 48. The medium was changed every 2 days.

Cytotoxicity assay

Confluent cultures of MRC-5 or AGS cells were treated with medium containing the sesquiterpenes as well as with the reference compound lansoprazole at concentrations of 0–1000 μM. The products were first dissolved in dimethyl sulfoxide (DMSO) and then in the corresponding culture medium supplemented with 2% FBS. The final content of DMSO in the test medium and controls was 1%. Cells were exposed for 24 h to the test medium with or without the compound (control). Each concentration was tested in quadruplicate together with the control and repeated three times in separate experiments. At the end of the incubation, the neutral red uptake (NRU) assay was carried out (Rodríguez & Haun 1999). To calculate the IC50 values (concentration that produces a 50% inhibitory effect on the evaluated parameter) the results were transformed to percentage of controls and the IC50 values were graphically obtained from the dose–response curves.

Lipophilicity

The lipophilicity of the compounds was calculated using the Chem Office 2002 version 8.0 software. The parameter is presented as log *P*.

Statistical analysis

Results were expressed as the mean ± s.d. In all experiments, statistical differences between several treatments and their respective control were determined by one-way analysis of variance and when the *F* value was significant, post-hoc differences were determined by the Dunnett's multiple comparison test. The level of significance was set at $P < 0.05$. All statistical analyses were performed using the software Statistica 5.1 (StatSoft, Inc.).

Results

Some 7 derivatives, including an alcohol, esters and amides, were prepared starting from sesquiterpene **3**. All the compounds were identified by spectroscopic means and compounds **5–8** were not previously reported in literature.

The ^{1}HNMR data of compounds **1–8** is presented in Table 1. Signals were assigned using COSY, HMBC and DEPT data. The ^{13}CNMR data of compounds **1–8** is presented in Table 2. All compounds prepared in this work exhibited spectroscopic data in agreement with the proposed structures. The chemical structures of the compounds are shown in Figure 1.

The TLC of compound **8** on silica showed a single spot in several solvent systems. In the ^{1}HNMR spectrum of compound **8**, most of the H signals appear duplicate with integrals in a 2:1 ratio. The most distinctive signals were the H-14 methyl group at δ 0.78 and δ 0.86 for the main and minor compound, respectively, as well as the signals assigned to H-4 and H-5 (Table 1), indicating a 2:1 mixture of isomers. The two products obtained as a result of hydrogenation is a consequence of the H addition at the double bond both from the upper and the lower side of the molecule. The *cis* orientation of the COOH and the H-14 methyl group explain the deshielding effect observed for the minor compound (H-14 CH_3 at δ 0.86) while the same methyl group appears at δ 0.78 in the *trans*-configured main compound. The derivative **8a** is more stable thermodynamically as well as kinetically and appears in a 2:1 ratio with the isomer **8b**. The differences in both structures are also present in the ^{13}CNMR data, where the C-4 and C-5 signals clearly differ, suggesting a different stereochemistry (Table 2). The compounds could not be isolated as pure compounds and were assessed as the diasteromeric mixture.

At doses of 25, 50 and 100 mg kg^{-1}, cyperenoic acid showed a dose-dependent gastroprotective effect, reducing the ulcers by 45 and 75% at 50 and 100 mg kg^{-1}, respectively, compared with the untreated controls (Table 3). From the sesquiterpenes **1–8**, assessed at a single oral dose of 50 mg kg^{-1}, the best gastroprotective effect was observed for derivative **8**, obtained as a diasteromeric mixture by reduction of the 4,5-double bond of cyperenoic acid. Compound **8** reduced the lesion index by 86%, being the most active of the sesquiterpenes evaluated in this work and more active than lansoprazole at 20 mg kg^{-1}. The products **1** and **3–8** did not show significant differences in gastroprotective activity. Cyperenol and cyperenoic acid methyl ester, however, were more cytotoxic with IC50 values of 44 and 75, 48 and 75 μM against AGS cells and fibroblasts, respectively. The best gastroprotective effect with lower cytotoxicity was found for the compound **8**, cyperenoic acid (compound **3**) and the *p*-anisidyl derivative **7** (Table 3).

Discussion

Cyperenol was first described as a constituent of the essential oil from the tubers of *Cyperus scariosus* (Nerali & Chakravati 1967). Cyperenyl acetate was reported from the root of *Cirsium dipsacolepis* (Takano & Kawaminami 1988). Cyperenoic acid has been reported from *Sandwithia guyanensis, Croton crassifolius* (Dictionary of Natural Products 2006) and *Jatropha isabellii* (Schmeda-Hirschmann et al 1996). Cyperenal, cyperenoic acid and its methyl ester were isolated from the root bark of the Brazilian Euphorbiaceae *Joannesia princeps* (Achenbach & Benirschke 1997).

Some biological actions have been described for cyperenoic acid derivatives. Achenbach & Benirschke (1994) reported the toxicity of cyperenol as stronger than that of podophyllotoxin towards the brine shrimp *Artemia salina*.

Table 1 ^{1}H NMR data of compounds **1–8** (400 MHz, $CDCl_3$, δ-values)

H	1	2	3	4	5	6	7	8a	8b
2	1.71 m;	1.66 m;	1.78 m;	1.76 m;	1.78 m;	1.70 m;	1.84 m;	1.62 m;	1.60 m;
	1.51 m	1.44 m	1.57 m	1.54 m	1.55 m	1.50 m	1.61 m	1.48 m	1.48 m
3	2.69 m;	2.59 m;	2.83 m;	2.82 m;	2.81 m;	2.75 m;	2.92 m;	2.23 m;	2.13 m;
	2.46 m	2.36 m	2.74 m	2.70 m	2.73 m	2.62 m	2.79 m	1.85 m	1.95 m
4	—	—	—	—	—	—	—	3.08 ddd	2.67 ddd
								(12.7, 11.0, 9.1)	(17.6, 11.0, 6.6)
5	—	—	—	—	—	—	—	2.81 ddd	2.54 ddd
								(12.5, 10.0, 7.3)	(15.7, 9.8, 5.9)
6	2.35 m;	2.34 m;	2.77 m;	2.76 m;	2.75 m;	2.64 m;	2.83 m;	1.86 m;	1.97 m;
	1.90 m	1.86 m	2.28 brd (16)	2.24 brd (16)	2.25 brd (16)	2.15 brd (16)	2.32 brd (16)	1.62 m	1.63 m
7	1.90 m	1.84 m	2.00 m	1.98 m	2.02 m	1.84 m	2.07 m	1.75 m	1.91 m
8	1.86 m;	1.87 m;	1.92 m;	1.90 m;	1.92 m;	1.93 m;	1.94 m;	1.84 m;	1.84 m;
	1.33 m	1.28 m	1.41 m	1.38 m	1.40 m	1.30 m	1.43 m	1.30 m	1.26 m
9	1.48 m;	1.42 m;	1.55 m;	1.52 m;	1.53 m;	1.45 m;	1.59 m;	1.55–1.65 m;	1.55–1.65 m;
	1.13 m	1.07 m	1.17 m	1.14 m	1.16 m	1.08 m	1.20 m	1.09 m	1.04 m
10	2.03 m	1.96 m	2.10 m	2.08 m	2.09 m	1.99 m	2.12 m	2.02 m	2.01 m
12	0.83 s	0.76 s	0.86 s	0.84 s	0.86 s	0.77 s	0.89 s	0.96 s	1.00 s
13	1.00 s	0.93 s	1.03 s	1.01 s	1.03 s	0.93 s	1.05 s	0.99 s	0.96 s
14	0.86 d	0.79 d	0.90 d	0.87 d	0.88 d	0.79 d	0.92 d	0.78 d	0.86 d
15	4.25 d (12.7);	4.62 d (12.2);	—	—	—	—	—	—	—
	4.21 d (12.7)	4.55 d (12.2)							
1′	—	2.04 s	—	3.75 s	3.40 m (7.3)	3.27 m	—	—	—
2′	—	—	—	—	1.22 t (7.3)	1.48 m	7.52 d (8.8)	—	—
3′	—	—	—	—	—	1.33 m	6.90 d (9.0)	—	—
4′	—	—	—	—	—	0.89 t (7.3)	—	—	—
5′	—	—	—	—	—	—	6.90 d (9.0)	—	—
6′	—	—	—	—	—	—	7.52 d (8.8)	—	—
7′	—	—	—	—	—	—	3.83 s	—	—
NH	—	—	—	—	5.46 br s	5.50 br s	7.17 br s	—	—

Compounds **1–8**: *J* 14, 10: 6.6 Hz.

Table 2 ^{13}C NMR data of compounds **1–8** (100 MHz, $CDCl_3$, δ-values)

C	1	2	3	4	5	6	7	8a	8b
1	65.96 s	66.05 s	68.23 s	67.80 s	67.77 s	67.76 s	68.18 s	60.54 s	59.87 s
2	26.14 t	26.01 t	25.74 t	25.73 t	25.76 t	25.73 t	25.78 t	26.43 t	26.26 t
3	37.85 t	38.15 t	36.33 t	36.68 t	36.78 t	36.79 t	36.91 t	33.67 t	34.02 t
4	131.17 s	126.42 s	123.19 s	123.33 s	126.08 s	126.21 s	126.41 s	47.72 d	52.74 d
5	146.27 s	148.83 s	173.10 s	169.30 s	165.55 s	165.60 s	163.63 s	43.54 d	46.98 d
6	27.58 t	27.53 t	31.34 t	31.08 t	30.67 t	30.64 t	30.88 t	30.69 t	33.57 t
7	48.56 d	48.51 d	48.20 d	48.20 d	48.61 d	48.61 d	48.70 d	47.24 d	48.95 d
8	27.61 t	27.60 t	26.96 t	27.01 t	27.11 t	27.09 t	27.08 t	27.15 t	27.18 t
9	28.13 t	28.07 t	27.90 t	27.89 t	27.90 t	27.89 t	27.93 t	29.54 t	28.42 t
10	35.33 d	35.34 d	36.00 d	35.86 d	35.78 d	35.76 d	35.92 d	32.43 d	33.51 d
11	41.18 s	41.22 s	41.73 s	41.63 s	41.48 s	41.45 s	41.61 s	44.43 s	44.10 s
12	26.12 q	26.01 q	26.22 q	26.19 q	26.20 q	26.18 q	26.24 q	27.30 q	27.98 q
13	19.29 q	19.27 q	19.28 q	19.26 q	19.23 q	19.20 q	19.24 q	20.96 q	20.46 q
14	17.95 q	17.87 q	17.99 q	17.93 q	17.91 q	17.89 q	17.98 q	17.57 q	16.89 q
15	60.62 t	61.92 t	171.05 s	166.00 s	161.73 s	161.45 s	155.19 s	180.75 s	182.82 s
1′	—	170.99 s	—	50.90 q	34.06 t	39.00 t	131.39 s	—	—
2′	—	20.91 q	—	—	15.10 q	31.91 t	114.16 d	—	—
3′	—	—	—	—	—	20.20 t	121.47 d	—	—
4′	—	—	—	—	—	13.77 q	157.35 s	—	—
5′	—	—	—	—	—	—	121.47 d	—	—
6′	—	—	—	—	—	—	114.16 d	—	—
7′	—	—	—	—	—	—	55.50 q	—	—

Table 3 Gastroprotective effect of cyperenoic acid (compound **3**) and its derivatives at 50 mg kg^{-1} and lansoprazole (20 mg kg^{-1}) on HCl/EtOH-induced gastric lesions in mice, lipophilicity and cytotoxicity towards AGS cells and human fibroblasts

Compound	Lipophilicity (Log P)	Lesion index (mm)	Lesion reduction (%)	Cytotoxicity IC50 (μM)	
				AGS	Fibroblasts
Compound **3**					
25 mg kg^{-1}		44.3 ± 6.7	0		
50 mg kg^{-1}		24.4 ± 7.3	45		
100 mg kg^{-1}		11.0 ± 5.0	75		
Control		44.3 ± 7.7			
Compound					
1 (50 mg kg^{-1})	3.156	8.3 ± 3.0**	77	44	75
2 (50 mg kg^{-1})	3.385	18.1 ± 3.8**	49	143	163
3 (50 mg kg^{-1})	3.287	13.4 ± 5.2**	62	463	968
4 (50 mg kg^{-1})	3.550	10.0 ± 3.3**	72	48	75
5 (50 mg kg^{-1})	3.210	8.9 ± 3.2**	75	189	474
6 (50 mg kg^{-1})	4.113	8.4 ± 3.2**	76	96	91
7 (50 mg kg^{-1})	4.409	12.7 ± 2.2**	64	439	841
8 (50 mg kg^{-1})	3.678	5.0 ± 2.2**	86	618	950
Lansoprazole	—	9.4 ± 3.3**	73	—	—
Control	—	35.4 ± 4.5	—	—	—

Results are expressed as means ± s.d., n = 8. *$P < 0.05$, **$P < 0.01$, compared with the controls (analysis of variance followed by Dunnett's test).

The gastroprotective effect of cyperenoic acid and seven derivatives was assessed in the HCl/EtOH-induced gastric lesion model in mice at 50 mg kg^{-1}. While the gastroprotective effect of the alcohol cyperenol was comparable with that of cyperenoic acid, acetylation of the OH function lowered the gastroprotective effect. There were no statistically significant differences in the gastroprotective effect of cyperenoic acid and the corresponding methyl ester but a strong increase in cytotoxicity was observed after methylation. The gastroprotective effect of cyperenol, cyperenoic acid methyl ester and the ethyl- and butylamides from cyperenoic acid were in the same range, reducing the gastric lesions by 72–77%. Acetylation of the alcohol function at C-15 strongly reduced the gastroprotective effect in our mouse model. The amides **5–7** presented similar effect in the gastroprotection assay. In compounds **5** and **6**, the length of the alkyl chain does not seem to play a relevant role in the gastroprotective effect, but increases cytotoxicity, as can be seen from the IC50 values. In spite of the better gastroprotective effect of patchoulan-15-oic acid (compound **8**) compared with that of cyperenoic acid (compound **3**) (86% vs 62% reduction of lesions), the values were not statistically different. There was, however, a distinctive difference in the cytotoxicity of compound **8** against AGS cells, with an IC50 of 618 μM instead of 463 μM for cyperenoic acid (compound **3**).

The cytotoxicity of the alcohol (compound **1**) was higher than that of the acetate (compound **2**) with IC50 values of 44 and 143 μM for AGS and 75 and 163 μM for fibroblasts, respectively. In the amides **5–7**, an increase in the alkyl side chain from C2 (compound **5**) to C4 (compound **6**) led to higher cytotoxicity in both cell lines. No clear relation was observed between the gastroprotective effect, cytotoxicity and lipophilicity of the assayed products.

Conclusions

This study presents evidence that the gastroprotective effect ascribed to the Paraguayan crude drug yaguá rová can be related, at least in part, to the occurrence of cyperenoic acid in the plant rhizomes. Additional studies should be undertaken, including the main diterpene jatrophone, present in the rhizomes. Since the best gastroprotective compound in this study was derivative **8**, obtained as a mixture of two isomers, selective synthesis using chiral reagents during hydrogenation should be undertaken to obtain the pure isomers. Biological evaluation of the pure isomers will make clear whether the activity observed is due to either **8a** or **8b** alone, or the synergistic effect of the mixture.

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