Antipyretic, Hypothermic and Antiinflammatory Activities and Metabolites from *Solanum ligustrinum* Lood.

C. Delporte,[1]* N. Backhouse,[1] R. Negrete,[1] P. Salinas,[1] P. Rivas,[1] B. K. Cassels[2] and A. San Feliciano[3]

[1]Laboratorio de Farmacognosia, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Casilla 233, 1-Santiago, Chile.
[2]Facultad de Ciencias, Universidad de Chile. Casilla 653, Santiago, Chile
[3]Facultad de Farmacia, Universidad de Salamanca, Avda. Campo Charro s/n, 37007, Salamanca, Spain

The acute toxicities of the global methanol extract of *Solanum ligustrinum* (Solanaceae) and the crude steroidal glycoalkaloids mixture were determined. The antipyretic, hypothermic and antiinflammatory activities of aqueous, global methanol, petroleum ether, dichloromethane and methanol extracts and crude steroidal glycoalkaloids mixture of the aerial parts were evaluated.

All the extracts and the crude steroidal glycoalkaloids mixture were inactive in the hypothermic activity assay, nevertheless, they showed antipyretic and antiinflammatory activities. Scopoletin and *β*-sitosterol 3-O-*β*-D-glucoside were isolated and identified from the dichloromethane extract. The impure coumarin fraction showed antipyretic and antiinflammatory activities and *β*-sitosterol glucoside exhibited antiinflammatory activity. In the light of the results of several NOE experiments, the H-5 and H-8 signals in the ^{1}H-NMR spectra of scopoletin were reassigned.

The methanol global extract yielded a crude steroidal glycoalkaloids mixture with antipyretic and antiinflammatory effects. © 1998 John Wiley & Sons, Ltd.

In Chile herbal remedies are used frequently to treat a large variety of ailments and symptoms, such as fever and inflammatory conditions, but little is known about the efficacy and innocuousness of these plants. *Solanum ligustrinum* is a native species which grows in central Chile and is used in folklore medicine for the treatment of fever as an infusion, decoction and macerate of the aerial part, without scientific data to support this use and its lack of toxicity (Montes and Wilkomirsky, 1987; Muñoz *et al.*, 1981). Steroidal glycoalkaloids such as solanine and the genins solasodine, tomatillidine and dihydrotomatillidine were isolated in earlier studies from this species (Bianchi *et al.*, 1960, 1961; 1965).

The literature describes antifungal properties and a certain degree of toxicity for steroidal glycoalkaloids found in species of *Solanum* (Keeler *et al.*, 1990; Roddick and Drysdale, 1984). It was demonstrated that aqueous macerated samples of *Solanum ligustrinum* inhibited the growth of *Candida albicans*, *Aspergillus niger* and *Achremonium falciforme* (Lazo, 1987; Giron *et al.*, 1988; Kusano *et al.*, 1987).

MATERIALS AND METHODS

General experimental procedures. Silica gel 60 was used for TLC and flash column chromatography (CC). TLC spots were detected under UV (254 and 365 nm), spraying with Dragendorff reagent and heating the plates to 110°C after spraying with Liebermann–Burchard (LB) reagent, $AlCl_3$ and NH_3. IR spectra (KBr disc) were recorded using a Perkin-Elmer 1310 infrared spectrometer. The NMR spectra were run in $CDCl_3$ or C_5D_5N solutions with TMS as an internal standard and recorded at 500 MHz (^{1}H) and 300/75 MHz (^{1}H/13C) in a Bruker spectrometer. The high resolution mass spectrum was recorded using a Kratos MS-50 spectrometer. The melting point was determined on a Kofler hot stage.

Plant material. *Solanum ligustrinum* Lood. Solanaceae was collected in the upper Maipo valley (Cajón del Maipo), SE of Santiago, latitude 34°S Chile, in early summer (December), and identified by Professor Carla Delporte. Voucher specimens are kept in the herbarium of the Escuela de Química y Farmacia, University of Chile (SQF: 17045).

Extraction and isolation. The dried and powdered material (1 kg) was extracted with methanol at room temperature. This global methanol extract (GME) (140 g)

was used for the acute toxicity studies and for the antiinflammatory and antipyretic assays, after removing the solvent under vacuum in a rotary evaporator. An aqueous extract or infusion (INFU), was prepared from dried and ground material, adding boiling water to a weighed amount, to obtain 20% and 40% (w/v) extracts for the antiinflammatory and antipyretic assays respectively. A decoction (DECO) was prepared by heating a weighed amount of plant material with water for 5 min, to obtain 20% and 40% (w/v) extracts. Macerates (MACE) were obtained at 20% and 40% w/v for the antiinflammatory and antipyretic assays respectively and were prepared with a weighed amount of the plant material with water and allowed to stand for the next 24 h.

As the GME showed antipyretic and antiinflammatory activities, and in order to continue with the chemical study, a new amount of *S. ligustrinum* (5 kg) was subjected to successive extractions with petroleum ether, dichloromethane and methanol, to afford the extracts PEE (110 g), DM (140 g) and ME (1200 g), respectively. All were subjected to antiinflammatory, hypothermic and antipyretic assays at doses of 600 mg/kg to monitor potential activities. The ME and DM extracts were submitted to CC eluting with mixtures of increasing polarity, and testing the fractions for bioactivity. Fractionation of ME (100 g) eluting with methanol–CH_2Cl_2 (50:50), afforded an alkaloid concentrate M-1 (30.5 g) giving an orange odour with Dragendorff reagent. Eluting with 100% methanol, a concentrate M-2 (8.5 g) rich in flavonoids was obtained, giving a positive reaction with NH_3 and $AlCl_3$.

The M-1 and M-2 fractions were submitted to successive fractionation in CC, yielding a crude steroidal glycoalkaloids mixture: CGA (20 g) when eluting with methanol: CH_2Cl_2 (40:60 and 50:50), and a flavonoid mixture: FLAV (8.5 g) when eluting with methanol: CH_2Cl_2 (90:10), respectively.

Fractionation of DM (70 g) eluting with hexane–CH_2Cl_2 (80:20), yielded a fraction (FSI) (5 g), rich in a compound S-1 accompanied by chlorophyll, showing an intense light blue fluorescence at 366 nm, and dark blue at 254 nm. Fractions eluted with hexane–CH_2Cl_2 (50:50) gave the concentrate named ImpS-2 containing a major component S-2 giving a purple colour with the LB reagent, which was submitted to successive fractionation in CC yielding S-2, purified by successive crystallization and identified for a complete spectral study.

Animals. Pirbright guinea-pigs (220–300 g) of both sexes were used for the antiinflammatory study and for antipyretic and hypothermic studies adult female New Zealand rabbits (2–3 kg) were used. The animals were kept under standard housing conditions at the Animal Maintenance Unit (UMA) of the Chilean Public Health Institute (ISP), and fasted overnight before the day of the experiments.

Acute toxicity. Groups of 10 CF-1 mice of either sex (20–25 g), were kept under laboratory conditions and allowed free access to water. GME and CGA, suspended in propylene glycol, were administered orally, via gastric catheter. Animals were observed and weighed daily for a week. The lethal dose 50 (LD_{50}) was calculated with the Morgan scoring method (1992).

Antipyretic activities. Antipyretic activity was deter-mined using three animals for each dose (modified from USP XXII, 1990) and repeating each experiment three times (nine results were obtained for each dose). Pyrexia was induced by i.v. injection of *Escherichia coli* endo-toxin (prepared in sterile saline) at a dose of 13 ng/kg. Rectal temperatures were recorded continuously for 180 min, with an Ellab Pyrogentester (model Z12DP) immediately after pyrogen injection.

The mean areas under curves temperature *vs* time obtained for each pyrogen-treated animal, with and without previous oral administration of the samples, were compared and the antipyretic effect (*E*) was calculated according to the following equation:

$$\%E = [1 - \text{area}_{\text{pyr+sample}} / \text{area}_{\text{pyr}}] \times 100$$

where $\text{area}_{\text{pyr+sample}}$ represents the mean area under the curve obtained after plotting temperature *vs* time in minutes for sample-tested rabbits, and area_{pyr} is the corresponding mean area for animals treated only with pyrogen. These mean areas were calculated for the two time intervals: from 0–90 and 90–180 min. The experi-ment was carried out in this way, since active principles with fast absorption and elimination could be present in the sample being studied, showing an effect only in the first 90 min of the assay. However, the active principles could have a slow absorption and the effect would only be seen after a 90–180 min interval (Delporte *et al.*, 1996). The mean areas were calculated using a computer program developed in our laboratory for this purpose.

Antiinflammatory activity. The antiinflammatory activ-ity was evaluated in groups of 10–15 animals for each dose, using the carrageenan-induced paw oedema described by Winter *et al.* (1963). Paw volume was measured with an Ugo Basile plethysmometer (model 7150) immediately, and 3 h after injecting 0.1 mL of sterile saline λ-carrageenan (1%). Antiinflammatory activity (% *A*) was evaluated as:

$$\%A = [(\%I_\text{c} - \%I_\text{s}) / \%I_\text{c}] \times 100$$

where % I_c is the mean inflammation reached in control guinea-pigs which received only the vehicle (37.7% ± 1.3% for 96 animals), and I_s is the average inflammation in drug-treated animals, expressed as:

$$\%I = [(V_\text{f} - V_\text{i}) / V_\text{i}] \times 100$$

where V_f and V_i are final and initial paw volumes, respectively, averaging % *I* over all the animals used in each test (Backhouse *et al.*, 1994).

In both assays, the samples were administered orally 1 h before the carrageenan or the endotoxin injection, by means of an intragastric catheter, suspended in propylene glycol. Sodium naproxen (SN) (from Laboratorios Saval, Chile) was used as a positive control and was suspended in the same vehicle. λ-carrageenan was purchased from Sigma. *E.coli* endotoxin was obtained from Chilean Health Institute (Backhouse *et al.*, 1994).

The significance of the drug-induced changes was estimated using the Wilcoxon test for independent data for the antiinflammatory assays and Wilcoxon tests for dependent data for antipyretic and hypothermic assays (Hollander and Wolfe, 1973). The Sm (SD/$\sqrt{n}$) values were calculated for mean % I_c and % I_s values and for the mean areas under the curve for treated and untreated animals in each assay.
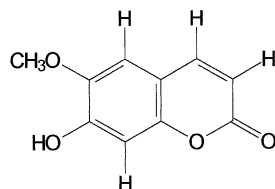
Figure 1. Scopoletin

Hypothermic activity. The hypothermic activity was evaluated in groups of three animals for each dose and each experiment repeated three times (nine results were obtained for each dose). Rectal temperatures were recorded continuously for 180 min, with an Ellab Pyrogentester (model Z12DP) immediately after the oral administration of the extracts studied. The mean areas under curve temperature *vs* time obtained for each with and without oral administration of the extracts were compared and the hypothermic effect (H) was calculated according to the following equation:

$$\%H = [1 - \text{area}_{\text{sample}}/\text{area}_{\text{normal}}] \times 100.$$

RESULTS AND DISCUSSION

Acute toxicity

GME and CGA showed no toxic effect at a dose of 2.4 and 8 g/kg in mice. Common side effects including, mild diarrhoea, loss of weight and depression were not recorded. The LD_{50} for each sample was not determined as no deaths were observed, and due to the consistency of the extracts, higher doses could not be administered. It is important to carry out toxicological studies in other animal species in order to demonstrate its innocuousness.

DM showed antipyretic and antiinflammatory activities and was selected for further study. Its fractionation led to the isolation of two pharmacologically active compounds S-1 (30 mg) and S-2 (100 mg). The first compound was identified as scopoletin by spectral comparison. It crystallized as needles from methanol, MP = 204°C, and presented a coumarin characteristic light blue intense fluorescence at UV 366 nm. The mass spectrum showed a M^+ at 192.4, which could correspond

Table 1. NOE based assignment of ^{1}H-NMR spectral data for scopoletin (S-1)

H	δ ppm (*mult.*, J in Hz)
3	6.30 (*d*, 9.5)
4	7.68 (*d*, 9.5)
5	6.85 (s)
8	6.90 (s)
OCH$_3$	3.95 (s)
OH	6.15 (s)

CDCl$_3$, TMS as int. standard

to both scopoletin (7-hydroxy-6-methoxycoumarin) and isoscopoletin (6-hydroxy-7-methoxycoumarin) according to bibliographic data (Aplin and Page, 1967).

The ^{1}H-NMR spectrum also showed similarity with those of scopoletin and isoscopoletin (Aplin and Page, 1967). The ^{1}H chemical shifts values (∂) did not allow us to differentiate both isomers. Signals for H-3 (6.30 ppm, doublet, alpha to the CO group), H-4 (7.68 ppm, doublet, beta to the CO group), OCH$_3$ (3.95 ppm) and OH (6.15 ppm) were easily assigned by comparison with bibliographic data. This was not the case for the signals appearing at 6.90 and 6.85 ppm, which may correspond to H-5 or H-8 indistinctly. It was necessary to carry out some NOE difference experiments to ascertain their assignment. On irradiation of the OCH$_3$ signal (3.95 ppm) a NOE was observed for the signal at 6.85 ppm. The same signal was enhanced by NOE on irradiating the signal at 7.68 ppm, which unequivocally corresponds to H-4. These results confirm both the location of the methoxyl group at C-6 and the correct assignment of H-5 (6.85 ppm) and H-8 (6.90 ppm) (Table 1 and Fig. 1), which differ from those performed previously (Aplin and Page, 1967).

S-2 was identified as β-sitosterol 3-O-β-D-glucoside by comparison with an authentic sample by IR and ^{1}H-NMR and ^{13}C-NMR. The ^{1}H and ^{13}C signals of the NMR spectra were assigned by comparison with the bibliographic data (Villar and Payá, 1981).

The antipyretic activity of sodium naproxen is dose-related and in our experiments the maximum effect at both time intervals (0–90 min: 51% and 90–180 min: 81.1%) was reached at a dose of 25 mg/kg (Delporte *et al.*, 1996). The antiinflammatory response to sodium naproxen is also a dose-related effect, with a maximum

Table 2. Antipyretic effect (% *E*) of different extracts from *Solanum ligustrinum* and sodium naproxen

		Area$_{\text{pyr + s}} \pm$ Sm / Area$_{\text{pyr}} \pm$ Sm		% Antipyretic effect	
Sample	Dose (mg/kg)	0 – 90 min	90 – 180 min	0–90 min	90–180
INFU	800	28.7 ± 3.5/26.6 ± 3.1	86 ± 9.8/89.3 ± 7.9	0	0
DECO	800	39.9 ± 3.4/53.1 ± 3.8	19.0 ± 1.7/21.7 ± 1.7	25.0[a]	0
MACE	800	38.8 ± 4.5/43.5 ± 4.9	116.5 ± 12.6/117.5 ± 12.5	13.0	0
GME	600	15.3 ± 1.2/21.0 ± 2.9	6.4 ± 1.9/58.5 ± 11.1	27.1[a]	0
PEE	600	21.6 ± 4.7/47.7 ± 4.4	21.6 ± 2.9/14.3 ± 1.8	54.7[a]	0
DM	600	13.8 ± 2.9/31.4 ± 3.9	26.9 ± 4.7/18.1 ± 2.3	56.1[a]	0
ME	600	1.6 ± 3.0/17.0 ± 4.1	13.3 ± 2.5/27.7 ± 3.0	90.6[a]	0
CGA	50	8.8 ± 0.7/37.3 ± 3.8	30.4 ± 4.9/39.2 ± 3.2	76.4[a]	0
FLAV	50	58.6 ± 4.1/59.3 ± 1.7	63.5 ± 3.1/64.2 ± 4.0	0	0
FS1	50	23.4 ± 2.1/59.3 ± 1.7	64.8 ± 2.1/63.4 ± 3.4	60.5[a]	0
SN	25	15.6 ± 2.9/31.8 ± 3.7	9.5 ± 1.2/50.5 ± 8.0	51.0[a]	81.1[a]

[a] $p \leq 0.05$
INFU infusion; DECO decoction; MACE macerate; GME global methanol extract. PEE, DM, ME petroleum ether, dichloromethane and methanol extracts; CGA crude steroidal glycoalkaloids mixture; FLA crude flavonoids mixture; FS1 fraction rich in scopoletin; SN sodium naproxen; Sm = SD/$\sqrt{n}$; *n* number of animals in each assay.

Table 3. Antiinflammatory effect (% *A*) of different extracts and metabolites from *Solanum ligustrinum* and sodium naproxen

Sample	Dose (mg/kg)	% *I* ± Sm	% Antiinflammatory effect	*n*
Control		37.7 ± 1.3	0	96
INFU	800	27.1 ± 2.8	28.1[a]	38
DECO	800	25.8 ± 9.02	31.6[a]	17
MACE	800	28.5 ± 1.9	24.2[a]	22
PEE	600	19.8 ± 2.0	47.5[a]	24
DM	600	31.7 ± 3.2	16.0[a]	23
ME	600	19.9 ± 2.4	48.5[a]	18
CGA	30	28.3 ± 2.0	24.8[a]	16
FS1	50	13.3 ± 1.4	65.0[a]	12
S-2	30	19.1 ± 5.7	49.3[a]	7
SN	4	17.1 ± 0.8	54.6[a]	15

[a] $p \leq 0.05$

%*I*, mean inflammation in sample-treated animals. INFU, infusion; MACE, macerate; PEE, DM, ME, petroleum ether, dichloromethane and methanol extracts; CGA, crude steroidal glycoalkaloids mixture; FS1, fraction rich in scopoletin; S-2, 3β-sitosterol-O-D-glucoside; SN, sodium naproxen; Sm = SD/$\sqrt{n}$. *n*, number of animals in each assay.

Table 4. Antiinflammatory effects (% *A*) of GME from *Solanum ligustrinum* at different dose and sodium naproxen

Dose (mg/kg)	%*I* ± Sm	% *A*	*n*
Control	37.7 ± 1.3	0	96
400	24.0 ± 2.3	36.3[a]	30
600	18.3 ± 1.5	51.3[a]	32
800	10.2 ± 1.3	73.0[a]	15
1000	25.1 ± 2.1	33.4[a]	18
SN 4	17.1 ± 0.80	54.6[a]	15

[a] $p \leq 0.05$

%*I*, mean inflammation in sample-treated animals. GME, global methanol extract; SN, sodium naproxen; Sm = SD/$\sqrt{n}$; *n*, number of animals in each assay.

effect at 4.0 mg/kg of 54.6% (Backhouse *et al.*, 1994) (Tables 2 and 3). INFU showed no antipyretic effect, but exhibited an antiinflammatory effect (28.1%) at a dose of 800 mg/kg in the first interval. DECO gave a 25% antipyretic and 24.2% antiinflammatory effect respectively at a dose of 800 mg/kg. MACE at a dose of 800 mg/kg showed no antipyretic effect, but exhibited a 24.2% antiinflammatory effect (Tables 2 and 3). Only DECO, of the aqueous extracts, presented an antipyretic effect and the highest antinflammatory effect, leading us to propose that the active principles would have been extracted in a major quantity when the plant material was heated with boiling water. Assays performed on GME showed a dose- related antiinflammatory effect with a maximum of 73% at 800 mg/kg and an antipyretic effect of 27.1% at 600 mg/kg (Tables 2 and 4). PEE exhibited 54.7% antipyretic and 47.5% antiinflammatory effects. DM gave 56.1% antipyretic and 16% antiinflammatory effects. ME showed 90.6% antipyretic and 19.9% antiinflammatory effects (Tables 2 and 3). As can be observed, all successive extracts showed both activities, possibly due to the inhibition of the release of prostaglandins by the active principles of these extracts, that could explain both effects observed (Kluger, 1991; Weissmann, 1987).

The CGA proved to have marked antipyretic (76.4%) and slight antiinflammatory (24.8%) activities at doses of 50 and 30 mg/kg respectively (Tables 2 and 3). These crude steroidal glycoalkaloids mixture would be respon-

sible in part for the effects shown by GME and ME, which gave an antiinflammatory and a stronger antipyretic effect for the latter. One of the components of this mixture is solanine, a glycoside of solasodine (Bianchi *et al.*, 1961). Solasodine exhibited antinflammatory effects in arthritis caused by kaolin, similar to that of cortisone at the same doses (0.25 mg/kg) (Müller-Dietz (1972). For this reason, solanine could be one of the active principles responsible for the pharmacological effects found.

The fraction rich in scopoletin showed both activities at doses of 50 mg/kg: 60% antipyretic and 60.5% antiinflammatory effects (Tables 2 and 3). Scopoletin must be one of the active principles of this species, since this simple coumarin inhibited prostaglandin synthetase, and its use as an antiinflammatory topical application has been reported (Hussein and Samuelsson, 1992). Besides, unsubstituted coumarin is antipyretic (Ritschel *et al.*, 1984). The steroid β-sitosterol 3-O-β-D-glucoside gave an antiinflammatory effect (49.3%) at 20 mg/kg (Table 3). This is another of the active principles isolated from DM. According to the literature, the simple genin, β-sitosterol itself, exhibited antiinflammatory and antipyretic activities at doses of 155.6 and 160 mg/kg respectively (Gupta *et al.*, 1980).

It is important to note that in the assays of the antipyretic activity, the effects were seen only at the first time interval for all the samples in study, differing from the positive control that presented an effect at both time intervals. This may reflect substantial pharmacokinetic differences between sodium naproxen and the active principles of *S. ligustrinum*. All the extracts studied showed no hypothermic effects, they were especially effective in lowering an elevated temperature but did not affect appreciably a normal temperature. In view of the results, the present study demonstrates that *S. ligustrinum* exerts significant antipyretic and antiinflammatory *in vivo* activities.

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