Active constituents isolated from *Psoralea glandulosa* L. with antiinflammatory and antipyretic activities

C. Nadine Backhouse [a,*], Carla L. Delporte [a], Rosa E. Negrete [a], Silvia Erazo [a], Alexandra Zuñiga [a], Alvaro Pinto [a], Bruce K. Cassels [b]

[a] *Laboratory of Pharmacognosy, Faculty of Pharmaceutical and Chemical Sciences, University of Chile, PO Box 233, 1-Santiago, Chile*
[b] *Chemistry Department, Faculty of Sciences, University of Chile, Santiago, Chile*

Abstract

The antiinflammatory and antipyretic activities of the petroleum ether extract (PEE), dichloromethane extract (DME) and methanol extract (ME1) of the aerial part of *Psoralea glandulosa* L. (Papilionaceae) were studied. The bioactivity-guided fractionation of the active extracts yielded the isolation of bakuchiol (Bk) from the petroleum ether as the active compound, cyclobakuchiols A and B (Cbk), and angelicin (Ang) from DME.

Keywords: Psoralea glandulosa; Bakuchiol; Cyclobakuchiols; Angelicin; Antiinflammatory; Antipyretic

1. Introduction

Antiinflammatory and antipyretic activities of the infusion and global methanol extract of the aerial part of *Psoralea glandulosa* L. (Papilionaceae) 'culén', have been recently reported (Backhouse et al., 1996). Infusion of *P. glandulosa* is used for the treatment of wounds, stomach ailments and fever (Muñoz et al., 1981). Earlier research on the secondary metabolites of this plant led to the isolation of two furanocoumarins: angelicin (Ang) and psoralen, together with drupanin methyl ester and bakuchiol (Bk) (Erazo et al., 1990) earlier isolated from *P. corylifolia* (Mehta et al., 1966). Recently the isolation of a mixture of cyclobakuchiols A and B (Cbk) has been reported (Backhouse et al., 1995). In addition, the antimicrobial activity of Bk against Gram positive bacteria have been reported (Erazo et al., 1997).

In search of the active metabolites responsible for the antiinflammatory and antipyretic activities reported for this plant a bioactivity-guided study of the petroleum ether extract (PEE), dichloromethane extract (DME)and methanol extract (ME1) was initiated.

2. Materials and methods

NMR spectra in $CDCl_3$ with TMS as internal standard were recorded at 300 and 75 MHz (^{13}C) on a Bruker spectrometer, using available pulse programs for DEPT. TLC spots were detected under UV (254 and 366 nm) and heating the plates to 110 °C after spraying with Liebermann–Burchard reactive.

2.1. Plant material

The aerial part of *P. glandulosa* L. was collected in the upper Maipo valley (Cajón del Maipo), SE of Santiago, 34° S Lat., Chile, in early summer (December), and identified by Professor Carla Delporte. Voucher specimen is kept in the Herbarium of the Escuela de Quimica y Farmacia (SQF: 17273).

2.2. Extraction and isolation

Air dried and ground plant material (4 kg) was extracted successively at room temperature with petrol, CH_2Cl_2 and MeOH yielding respectively and after removal of the solvents in vacuo 80, 98 and 300 g of each extract respectively. All three extracts were submitted to antiinflammatory and antipyretic assays in guinea pigs and in rabbits respectively, to monitor potential activi-

ties. The DME (CH_2Cl_2 extract) proved to be the most active and therefore it was submitted to CC on silica gel, eluting with petrol and petrol–CH_2Cl_2 mixtures of increasing polarity, being bioactivity-guided. Two anti-inflammatory active fractions were obtained, one (B2, 20.0 g) eluted with petrol and the other (B7, 3.0 g) with petrol–CH_2Cl_2 (95:5). The first fraction B2 was purified by further dry CC on silica gel, yielding a mixture of Cbk [**1**, **2**] eluted with petrol–CH_2Cl_2 (86:14 to 82:18) (Backhouse et al., 1995). The active fraction B7 was purified in CC on silica gel eluted with petrol–CH_2Cl_2 (95:5), yielding 1.5 g of a furanocoumarin identified as Ang [**3**] by a complete spectral analysis. This furanocoumarin was submitted to antiinflammatory assays.

The PEE was fractionated by MPLC on silica gel, eluted with a mixture of petroleum ether–CH_2Cl_2 (8:2), isolating the most abundant compound identified as Bk [**4**] by spectroscopic analysis (Erazo et al., 1990). This meroterpenoid was submitted to antiinflammatory and antipyretic assays.

In this work, the ME1 was not fractionated due to its weak antiinflammatory activity.

2.3. Animals

Pirbright guinea pigs (200–300 g) of both sexes and adult female New Zealand rabbits were used for the antiinflammatory and antipyretic studies, respectively. The animals were kept under standard housing conditions at the Animal Mantainment Unit (UMA) of the Instituto de Salud Pblica (ISP), and fasted overnight before the day of the experiments.

2.3.1. Antiinflammatory activity

The antiinflammatory activity was evaluated in guinea pigs, in groups of 10–14 animals for each dose, using the carrageenan-induced paw edema described by Winter et al., 1963. Paw volume was measured with an Ugo Basile plethysmometer (model 7150) initially, and 3 h after injecting 0.1 ml of sterile saline λ-carrageenan (1%). Antiinflammatory activity (%A) was evaluated as:

$$\%A = \frac{(\%I_c - \%I_d)}{\%I_c} \times 100$$

where $\%I_c$ is the mean inflammation reached in control guinea pigs which have received only the vehicle ($37.7 \pm 1.3\%$ paw volume increase for a group of 96 animals), and I_d is the average inflammation in drug-treated animals, expressed as:

$$\%I_d = \frac{(V_f - V_i)}{V_i} \times 100$$

where V_f and V_i are final and initial paw volumes, respectively, averaging $\%I$ over all the animals used in each test. The significance of the drug-induced changes was estimated using Student's t-test, considering $P \leq 0.05$. The results are expressed as mean ± S.E.M.

2.3.2. Antipyretic activity

Antipyretic activity was determined in rabbits using three animals for each dose (modified from USP XXII) and repeating each experiment three times at intervals not less than two weeks. Pyrexia was induced by i.v. injection of *Escherichia coli* endotoxin (prepared in sterile saline) at a dose of 13 ng/kg. Rectal temperatures were recorded continously for 180 min, with an Ellab Pyrogentester (model Z12DP) immediately after pyrogen injection. The areas under temperature versus time curves obtained for each pyrogen-treated animal with and without earlier oral administration of the samples, were compared and the antipyretic effect (E) was calculated according to the following equation:

$$\%E = \frac{1 - \text{area}_{\text{pyr + drug}}}{\text{area}_{\text{pyr}}} \times 100$$

where $\text{area}_{\text{pyr + drug}}$ represents the area under the curve obtained after plotting temperature versus time in min for drug-tested rabbits, and area_{pyr} is the corresponding area for animals treated only with pyrogen. These areas were calculated for the two time intervals: from 0 to 90 min and 90 to 180 min, using a computer program developed in our laboratory for this purpose, and the significance of the effect as estimated using the analysis of variance (ANOVA) test. The results are expressed as mean ± S.E.M.

In both assays, the sample were administered orally 1 h before the carrageenan or the endotoxin injection, by means of an intragastric catheter, suspended in glycolpropylene. Sodium naproxen (SN) (donated by 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 at Instituto de Salud Pública, Chile.

3. Results

Antiinflammatory and antipyretic assays performed on PEE, DME and ME1 (Figs. 1 and 2 respectively) from the aerial part of the plant in comparisson with SN effects led us to continue our study with petrol and DME (64.5 and 71.6% antiinflammatory effect respectively; 21.7 and 54.1% of antipyretic effect respectively, at the dose of 600 mg/kg in both cases) for phytochemical studies. Bioactivity-guided chromatography of the petroleum extract resulted in the isolation of Bk, identified through 1D ^1H and ^{13}C-NMR spectral analysis, and compared with literature data (Erazo et al., 1990). Bk showed a dose related antiinflammatory effect, with a maximum effect of 45.6% at 12 mg/kg (Fig. 3), lower than the maximum effect of SN (54.6% at 4.0 mg/kg). Fig. 4 shows that the antipyretic effect of this meroterpenoid is obtained only in the first interval (0–90 min),

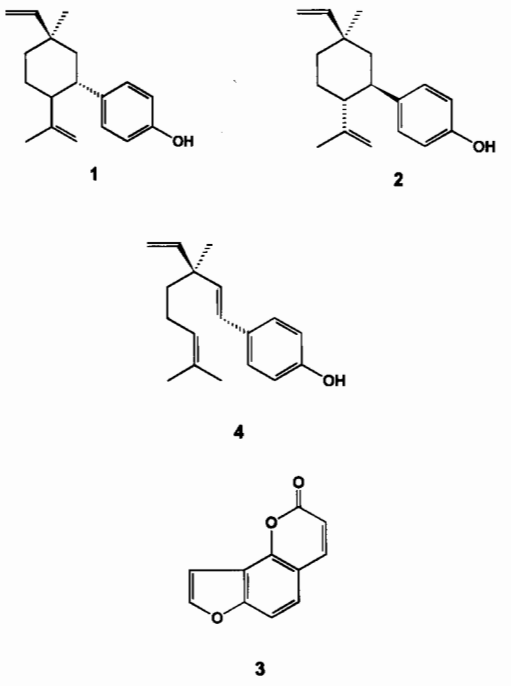

Fig. 1. Constituents isolated from *P. glandulosa* L.

at the dose of 20 mg/kg, corresponding to the effect of SN at 10 mg/kg.

Bioactivity-guided chromatography of the CH_2Cl_2 led us to the isolation of two active fractions, the first one, a mixture of Cbk (Backhouse et al., 1995) showed a 61.4% of antiinflammatory effect at the dose of 30 mg/kg (the antipyretic activity was not assayed, due to the small amount yielded); the second active fraction (3.0 g) eluted with petrol–CH_2Cl_2 (19:1) showed one major constituent on TLC (silica gel; $CHCl_3$; R_f 0.4 light blue fluorescence at 366 nm) that was purified by further crystallization and identified as Ang (1.5 g), an angular furocoumarin by a spectral analysis (Table 1). This coumarin was submitted to antiinflammatory and antipyretic assays. At the dose of 20 mg/kg, Ang showed a 39.0% of antiinflammatory effect (Fig. 3) and at the dose of 17 mg/kg a 68% of antipyretic effect (Fig. 4) in the first time interval.

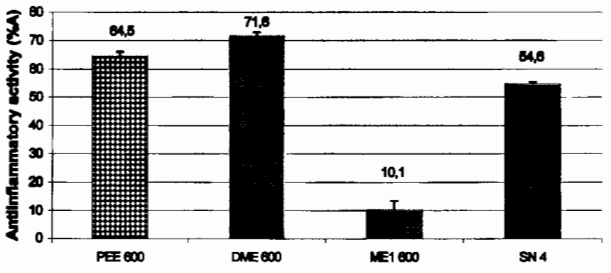

Fig. 2. Antiinflammatorv effects (%*A*) of PEE, DME and ME1 extracts from *P. glandulosa* and SN in guinea pigs. Abscissa shows the extract with a dose (mg/kg, p.o.). Results expressed as the mean ± S.E.M ($n = 10–14$).

4. Discussion

The antipyretic activity observed on the PEE (21.7% in the first interval) can be due to the presence of Bk, though the effect of the pure substance (20.4%) at 20 mg/kg is nearly equal to that of the extract at the dose of 600 mg/kg, and much weaker than the maximum effect observed for SN (51.0 and 81.1% in the first and second interval respectively). On the other hand, the effect of the PEE and Bk was only observed in the first 90 min of the assay, while in the case of SN this effect was greater in the second 90 min interval, which can reflect pharmacokinetic differences between these substances (Fig. 5).

Also, it can be appreciated that the antiinflammatory effect of the PEE at 600 mg/kg is higher than the maximum effect observed for Bk. Probably this compound is responsable of both activities, which could be due to the inhibition of the prostaglandin synthesis that are enhanced in inflammation (Samuelsson, 1987) and fever process (Saper and Breder, 1994).

From DME, two active compounds were obtained. Though, the effect of both Ang and Cbk can not be equally compared as their activity was assayed at different doses 20 and 30 mg/kg respectively, it can be observed that the cyclobakuchiols effect (61.4%) is much higher than that observed for Ang (39.0%), and the first is also higher than the maximum effect of SN (54.6%). DME showed higher antiinflammatory than antipyretic effect (71.6 and 54.1%, respectively) at equal dose.

In relation to the antiinflammatory effect of cyclobakuchiols in comparison with its biogenetic precursor, Bk, it seems to be that the cyclization of the molecule increases the antiinflammatory activity, as 45.6% is the maximum effect of the first at 12 mg/kg in comparison with a 61.4% of effect observed for the mixture of cyclobakuchiols at 30 mg/kg. By the other hand, antiinflamatory in vitro evaluations of Bk showed that this meroterpenoid is an agent able to control leukocytic functions such as eicosanoid production, migration and degranulation in the inflammatory site (Ferrándiz et al., 1996). Also, Bk has exerted a strong inhibition on DNA polymerase, and may be considered as a potential agent for use in chemotherapy (Sun et al., 1998). Other properties have also been described for this compound (Gordaliza et al., 1999). On the other hand, existing investigations in relation to the bioactivity of coumarins seems to indicate that several of these compounds exhibit antiinflammatory (Kosuge et al., 1985) and antipyretic (Lee et al., 1981) activities, in which the inhibition of prostaglandin syntetase has been proposed (Ritschel et al., 1982). Nevertheless, the antiinflammatory and antipyretic activities of Ang or any other angular furanocoumarin had not been earlier reported.

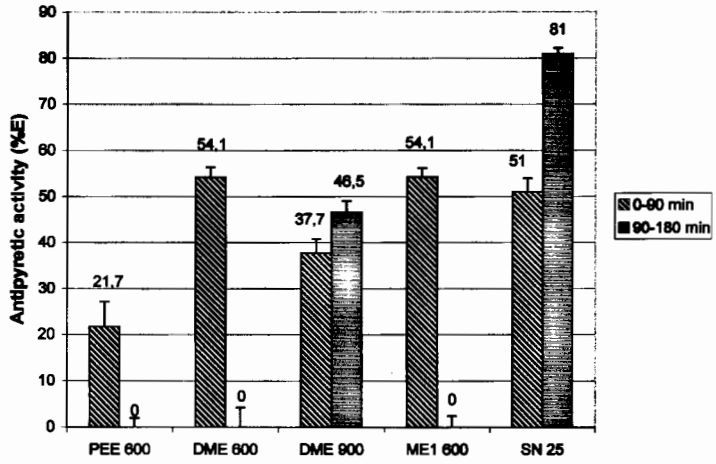

Fig. 3. Antipyretic effects (%E) of PEE, DME and MEl from from *P. glandulosa* and SN in rabbits. Abbreviation is the same as Fig. 2 ($n = 9$).

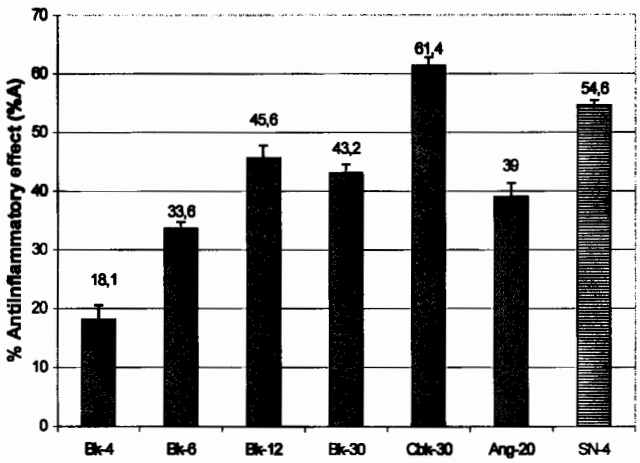

Fig. 4. Antiinflammatory effects of Bk, Cbk, Ang and SN in guinea pigs. Abscissa shows the constituent with a dose (mg/kg, p.o.). Results expressed as the mean ± S.E.M. ($n = 10$–14).

The results obtained in this research, may justify the potental use of the plant as antiinflammatory and antipyretic, giving it a partial scientific support to our traditional medicine. Also, Bk and cyclobakuchiols could be considered for future studies as possible new drugs for the treatment of these deseases. Alternatively, it is important to mention that Ang is less phototoxic against virus, bacteria and mammals than linear coumarins, if it is considered for the possible treatment of fever (Berenbaum and Feeney, 1981).

Table 1
1D NMR spectral data (300/75 MHz) for Angelicin

C	CH_n	δ_C	δ_H	J_{HH} (Hz)
2	C	160.8		
3	CH	114.1	6.37	9.6*d*
4	CH	144.6	7.79	9.6*d* 0.8*d*
5	CH	123.9	7.36	8.6*d*
6	CH	108.8	7.41	8.6*d* 0.8*d*
7	C	157.4		
8	C	116.9		
9	C	148.5		
10	C	113.5		
2′	CH	145.9	7.68	2.1*d*
3′	CH	104.1	6.37	2.1*d*

Data from ^1H, ^{13}C, DEPT spectra ($CDCl_3$), TMS as internal standard.

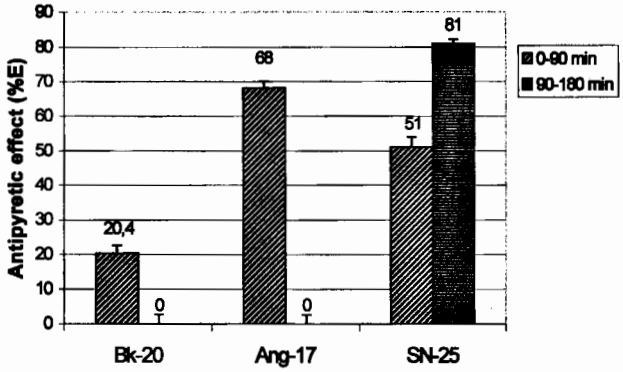

Fig. 5. Antipyretic effects of Bk, Cbk, Ang and SN in rabbits. Abbreviation is the same as Fig. 3 ($n = 9$).

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