



Antimycobacterial flavones from *Haplopappus sonorensis*

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Abstract

Crude extracts of *Haplopappus sonorensis* (A. Gray) S.F. Blake (Asteraceae), showed activity against *Mycobacterium tuberculosis* H₃₇Rv. By assay-guided fractionation, 5-hydroxy-3,7,4'-trimethoxyflavone (**1**), 5,7-dihydroxy-3,4'-dimethoxyflavone (**2**) and 5,4'-dihydroxy-3,7-dimethoxyflavone (**3**) were identified as the antimycobacterial principles. Compound **2** was the most active compound.

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1. Introduction

As part of a search for antimycobacterial compounds from higher plants from Baja California Sur (Mexico), we screened crude ethanolic extracts of plants used against infectious diseases. The screening revealed that *Haplopappus sonorensis* is active against *Mycobacterium tuberculosis* H₃₇Rv [1]. *H. sonorensis* or 'hierba del

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pasmo', a widely used plant in the northwest of Mexico, is claimed to cure 'pasmo', an illness described by symptoms such as bodily shaking, fever and cold. It is also used for treatment of skin ulcers, toothache, cough, cold, hurts, heart troubles, headache, rheumatism and sting of venomous animals [2,3].

The plant has been known under different taxonomic synonyms such as *Ericameria diffusa*, *Solidago diffusa*, *Linosyris sonorensis*, *Bigelovia diffusa*, *Aster sonoriensis* and *Chrysoma diffusa* [4]. Previous studies have also indicated that ethanolic extracts of *H. sonorensis* inhibit Gram (+) bacteria, as well as the spontaneous contractility of smooth muscle [5,6]. Thirteen flavonol methyl ethers, friedelan-3 α -ol and friedelin have been described from *H. sonorensis* [7–9]. The present study was undertaken with the aim of revealing the molecular explanation for the antimycobacterial activity of *H. sonorensis*.

2. Experimental

2.1. Plant material

The whole plant was collected in Cabo Pulmo, near La Paz, BCS, Mexico in August 1988. Jorge Agúndez, Fanerogamic Herbarium, Universidad Autónoma de Baja California Sur, is responsible for the taxonomic identification. Voucher specimens (No. 46-T) are deposited in the herbarium of the Pharmacognostic Research Program, UABCS (La Paz, BCS, Mexico).

2.2. Extraction

Dried and ground plant material (1.56 kg) was successively soxhlet-extracted with light petroleum, CH₂Cl₂ and EtOH. Each extract was concentrated in vacuo to dryness yielding: 3.37, 7.61 and 4.31% (w/w), respectively. Antimycobacterial and cytotoxic screening of the extracts led to further investigate the CH₂Cl₂ and light petroleum extracts.

2.3. Antimycobacterial bioassay

Antimycobacterial activity was determined against *M. tuberculosis* H₃₇Rv (ATCC 27294) by the BACTEC 460 system. Extracts, fractions and isolated compounds were tested at 100 μ g/ml in DMSO. Rifampin was included as a positive control [10].

2.4. Cytotoxicity test

Crude extracts, fractions and isolated compounds were evaluated against human colorectal cancer cells, HCT-116 (ATCC CCL-247). The cells were cultured in McCoy's 5A medium, supplemented with 10% fetal bovine serum. Samples dissolved in DMSO were added. The cells were incubated at 37 °C in an atmosphere of 5% CO₂ in air. At the end of the incubation period, cells were fixed and stained. Etoposide was included as a positive control. Median inhibitory concentration (IC₅₀) values were determined.

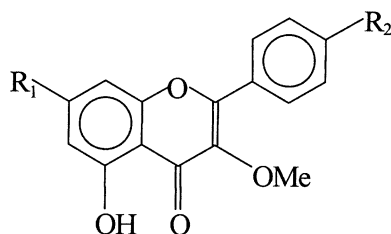


Fig. 1. 5-Hydroxy-3,7,4'-trimethoxyflavone (**1**); 5,7-dihydroxy-3,4'-dimethoxyflavone (ermanin) (**2**); 5,4'-dihydroxy-3,7-dimethoxyflavone (kumatakenin) (**3**).

3. Results and discussion

The CH_2Cl_2 and the light petroleum extracts show 60% inhibition against *M. tuberculosis* at 100 $\mu\text{g/ml}$, and an IC_{50} value of 20 $\mu\text{g/ml}$ toward the HCT-116 line. The EtOH extract was inactive. The CH_2Cl_2 extract of *H. sonorensis* was eluted on Si-gel CC with hexane–EtOAc gradients to afford 10 fractions. Fraction 6 displayed 88% inhibition against *M. tuberculosis* at 100 $\mu\text{g/ml}$, and an IC_{50} value of 9.8 $\mu\text{g/ml}$ in the HCT-116 cell line assay. Fraction 6, which was found to be the most active was further separated on Si-gel CC with CHCl_3 –EtOAc gradients to afford nine subfractions. Subfraction 3 had the highest activity against *M. tuberculosis*. Following ODS-CC, subfraction 3 gave compound **1** (12.1 mg; 0.0013% w/w), and a mixture of two flavones that yielded on HPLC compound **2** (6.8 mg; 0.00096% w/w) and compound **3** (8.6 mg; 0.0012% w/w) Fig. 1.

Compound **1** was identified by X-ray diffraction [8] as 5-hydroxy-3,7,4'-trimethoxyflavone, which was previously isolated from this source. Compounds **2** and **3** were identified as 5,7-dihydroxy-3,4'-dimethoxyflavone, and 5,4'-dihydroxy-3,7-dimethoxyflavone, respectively. Identification was performed by comparison of ^1H - and ^{13}C -NMR data with the literature [11]. Compound **2** and **3**, trivially named ermanin and kumatakenin were first isolated from *Betula ermani* [12], and *Alpinia kumatate* [13] although they, later on, have been encountered in many other plants.

Ermanin which has been reported to be antiviral [14,15], anti-inflammatory [16] and cytotoxic [17] was the most active compound against *M. tuberculosis*, showing 98% inhibition at 100 $\mu\text{g/ml}$. Ermanin is structurally related to nevadensine, a well known antimycobacterial flavone [18]. Both flavones have similar substitution patterns with 5,7-dihydroxy and 4'-methoxy substituents, but nevadensine is additionally methoxylated at C-6 and C-8, instead of at C-3 as ermanin. Compounds **1** and **3** were less active against *M. tuberculosis* showing 33 and 48% at the same concentration. Though ermanin was found to be a potent cytotoxic agent against human fibrosarcoma and murine colon carcinoma cells [17], we did not observe inhibition of HCT-116 cells by ermanin, or compound **1**. Kumatakenin displayed low cytotoxicity against the HCT-116 cell line with an IC_{50} value of 80.9 $\mu\text{g/ml}$. Kumatakenin was reported as an antiulcer [19], and antiviral drug [14].

The light petroleum extract was treated with a mixture of light petroleum/Me₂CO (9:1) to afford **1**. The supernatant was concentrated until dryness, and treated again with Me₂CO. The Me₂CO fraction was separated by Si-gel CC using a hexane–toluene–Me₂CO gradient as eluent, to give 11 fractions. Fraction 6 was the most active against *M. tuberculosis* (98% of inhibition at 100 µg/ml). Detailed TLC and HPLC analysis of this fraction, under different conditions, revealed the presence of ermanin **2** and kumatakenin **3** as major constituents. Chromatographic comparisons were done using authenticated standards. Because we have found the same active compounds in the most active fraction of both lipophilic extracts, we believe that 5-hydroxy-3,7,4'-trimethoxyflavone **1**, ermanin **2** and kumatakenin **3** are the main antimycobacterial compounds in *H. sonorensis*.

These data, together with those previously reported, seem to confirm the uses and effects of herbal preparations of *H. sonorensis*.

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