2017

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Antibacterial activity of fractions from three Chumash medicinal plant extracts and *in vitro* inhibition of the enzyme enoyl reductase by the flavonoid jaceosidin

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Antibacterial activity of fractions from three Chumash medicinal plant extracts and *in vitro* inhibition of the enzyme enoyl reductase by the flavonoid jaceosidin

We have investigated the *in vitro* antibacterial bioactivity of dichloromethane-soluble fractions of *Artemisia californica*, *Trichostema lanatum*, *Salvia apiana*, *Sambucus nigra* ssp. *cerulea* and *Quercus agrifolia* Née against a Δ*tolC* mutant strain of *E. coli*. These plants are traditional medicinal plants of the Chumash Native Americans of Southern California. Bioassay-guided fractionation led to the isolation of three flavonoid compounds from *A. californica*: jaceosidin (**1**), jaceidin (**2**), and chrysoplenol B (**3**). Compounds **1** and **2** exhibited anti-bacterial activity against *E. coli* Δ*tolC* in liquid cultures. The *in vitro* activity of **1** against the enoyl reductase enzyme (FabI) was measured using a spectrophotometric assay and found to completely inhibit FabI activity at a concentration of 100 μM. However, comparison of MIC values for **1-3** against *E. coli* Δ*tolC* and an equivalent strain containing a plasmid constitutively expressing *fabI* did not reveal any selectivity for FabI *in vivo*.

Keywords: *Artemisia californica*, enoyl reductase, jaceosidin, Native American plants, antimicrobial, flavonoids

1. Introduction

Fatty acids are essential molecules in all organisms and there are two types of biosynthetic mechanisms for producing fatty acids in nature. Type 1 fatty acid synthase (FAS I) is a multifunctional enzyme complex found in animals and plants that contains all of the machinery needed to make fatty acids in a single multi-enzyme complex (Chirala & Wakil 2004). In contrast to this unified system, bacteria use the Type 2 fatty acid synthase pathway (FAS II) to synthesize fatty acids which is composed of discrete enzymes that produce fatty acids in a series of individual reactions and is considered to be an excellent focus for antibacterial drug discovery (Parsons & Rock 2011; Wang & Ma 2013).

Previous studies of the antibacterial properties of plant metabolites have demonstrated that many polyphenolic and terpenoid compounds from plants inhibit bacterial FAS II enzymes (Zhang & Rock 2004; Tasdemir et al. 2006). These findings strongly support the idea that targeting fatty acid biosynthesis in general, and the FASII enzyme enoyl reductase (FabI in

Escherichia coli) in particular, may be promising strategies for the development of new antibiotics (Parsons & Rock 2011; Wang & Ma 2013). The purpose of the study was to investigate the antibacterial properties of some traditional medicinal plants of the Chumash Native Americans and identify compounds from these plants that inhibit the enzyme FabI. We screened fractions from a panel of these plants for antibacterial activity and we describe the isolation of three flavonoid compounds (**1**-**3**) with antibacterial activity from *Artemisia californica*. The flavonoid jaceosidin (**1**) exhibited *in vitro* inhibitory activity against FabI but no selectivity against the enzyme was observed *in vivo*.

2. Results and Discussion

A group of plants native to the coastal region of Southern California were selected for screening of their potential antibacterial properties based on documented reports of their traditional use as medicinal treatments by the Chumash people, a native tribal group indigenous to the coastal region of Southern California in the United States (Timbrook 2007). The plants selected for this study were *Artemisia californica* Less. (Asteraceae), *Trichostema lanatum* Benth. (Lamiaceae), *Salvia apiana* Jeps. (Lamiaceae), *Sambucus nigra* ssp. *cerulea* (Adoxaceae) and *Quercus agrifolia* Née (Fagaceae) (Table S1). Two of the plants that were screened also belong to genera (*Artemisia* and *Salvia*) with long traditions of medicinal use in cultures throughout the world (Tan et al. 1998; Wu et al. 2012).

Leaves and stems from each plant specimen were collected in Malibu, California and the dichloromethane-soluble (DCM) fraction of each extract was generated as described in the Supplementary Materials. The DCM fractions were screened for antibacterial activity against a Δ*tolC* mutant strain of the gram-negative bacterium *E. coli*. The *tolC* knockout mutant reduces the activity of TolC-dependent multidrug efflux pumps that bind foreign molecules such as antibiotics and move them out of the cell, thus protecting the bacterium from poisonous molecules (Jackowski et al. 2002). The DCM fractions of *S. nigra* ssp. *cerulea* and *Q. agrifolia* did not exhibit any antibacterial activity, but fractions of *A. californica*, *T. lanatum* and *S. apiana* inhibited the growth of *E. coli* Δ*tolC* (Table S1).

Based on the results of the initial screening, the DCM fraction from *A. californica* was further fractionated by reverse-phase HPLC and bioassay-guided fractionation was used to

isolate the flavonoids jaceosidin (**1**), jaceidin (**2**), and chrysoplenol B (**3**) (Figure 1). Comparison of ^{1}H and ^{13}C NMR spectra to published values confirmed the identity of these compounds (Martínez et al. 1987; Sy & Brown 1998; Flamini et al. 2001; Huong et al. 2005). In light of other studies that have demonstrated that flavonoids and other plant polyphenols are effective inhibitors of FabI (Zhang & Rock 2004), a two-stage bioassay was developed to compare the activity of **1**-**3** against the *E. coli* Δ*tolC* control strain and against the same strain engineered to constitutively express the *fabI* gene from a plasmid (*E. coli* Δ*tolC* [*fabI*]). Decreased growth inhibition of the [*fabI*] strain compared to the control strain is an indicator that samples may be inhibitors of the FabI enzyme since excess amounts of FabI reduce the antimicrobial effect of active samples (Heath et al. 1998). None of the isolated compounds demonstrated greater potency against the Δ*tolC* control strain compared to the [*fabI*] strain (Table 1). The minimum inhibitory concentration (MIC) for **1** and **2** against both strains was 10 μM and 50 μM, respectively, while **3** did not exhibit any significant inhibitory activity. These results suggested that that these compounds did not inhibit FabI *in vivo*. All three compounds were also tested for inhibitory activity against a wild-type strain of *E. coli* which did not contain the *tolC* mutation; no inhibition of the *E. coli* WT strain was observed upon treatment with **1-3** at concentrations of up to 100 μM. All three *E. coli* strains were also treated with triclosan as a positive control. The large increase in the MIC of triclosan against the [*fabI*] strain (0.025 μM) compared to the Δ*tolC* strain (0.007 μM) showed that compounds that inhibit FabI exhibit decreased potency in the [*fabI*] strain, in accordance with other reports (Heath et al. 1998).

Although **1-3** did not exhibit any apparent selectivity against FabI *in vivo*, **1** was tested for FabI inhibitory activity using an *in vitro* assay (Figure 2) since other reports suggest that many flavonoids are effective inhibitors of FabI (Zhang & Rock 2004; Tasdemir et al. 2006). Compounds **2** and **3** were not tested in this assay due to a lack of materials. Inhibition of FabI activity by **1** was complete at a concentration of 100 μM and the estimated IC_{50} value was 75 μM. Inhibition of FabI with triclosan (30 μM) as a positive control resulted in only 30% of the activity of the uninhibited enzyme (Figure 2). These results, in combination with the absence of any decreased sensitivity to jaceosidin in *E. coli* Δ*tolC* [*fabI*] (Table 1, Figure S8), provide strong evidence that although **1** does inhibit FabI, its full antibacterial properties probably arise from more than one mechanism of action since the MIC for **1** was lower than the concentrations used to inhibit FabI *in vitro*.

Compound **1** has been previously identified in other species in the Asteracae family and has been reported to possess anti-inflammatory (Clavin et al. 2007), antibacterial (Barnes et al. 2013) and antiallergenic (Min et al. 2009) activity as well as activity in a variety of other bioassays related to cell signaling (Goettert et al. 2010; Nam et al. 2013; Lee et al. 2013). However, this study is the first time it has been identified in *A. californica* and it is the first report of its inhibitory activity against enzymes in the FASII pathway. Our findings in this study corroborate the conclusions of other studies that although plant polyphenol compounds can inhibit the FabI enzyme it is likely not their only mechanism of inhibiting bacterial growth (Zhang & Rock 2004).

3. Conclusions

In this study, extracts from a panel of Native American traditional medicinal plants were screened for antibacterial activity. We found that DCM fractions from *A. californica*, *T. lanatum* and *S. apiana* inhibited the growth of *E. coli* Δ*tolC*. Bioassay-guided fractionation of the DCM fraction from *A. californica* led to the isolation of three flavonoid compounds: jaceosidin (**1**), jaceidin (**2**), and chrysoplenol B (**3**). The flavonoid **1** exhibited *in vitro* inhibitory activity against the enoyl reductase enzyme (FabI) with an estimated IC_{50} of 75 μM. However, comparison of MICs for **1-3** against an *E. coli* Δ*tolC* control strain and an equivalent strain containing a plasmid constitutively expressing *fabI* (10 μM, 50 μM and >100 μM for **1, 2** and **3**, respectively, in both strains) did not reveal any selectivity for FabI *in vivo*. Although the compounds identified in this study are not new, this is the first reputable report of any flavonoids from *A. californica*. Additionally, these findings support the proposal that while flavonoids may inhibit enoyl reductase enzymes their antibacterial activities probably arise from more than one mechanism of action.

Supplementary Material

Experimental methods with ^{1}H and ^{13}C NMR spectra of **1-3** and bacterial growth inhibition curves are available in the Supplementary Material.

Acknowledgements

We would like to thank Thomas Huggins and Adilene Barajas for assistance in collecting specimens for deposit in the University of California, Los Angeles Herbarium and Charles O. Rock for providing the *E. coli* Δ*tolC* strains. This work was supported with funding from Seaver College and the Natural Science Division of Pepperdine University.

Conflicts of Interest

The authors declare no conflict of interest.

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Table 1. Summary of MIC data for flavonoids from *A. californica*

Bacterial strain	triclosan	**1**	**2**	**3**
E. coli WT	0.100 μM	>100 μM	>100 μM	>100 μM
E. coli Δ*tolC*	0.007 μM	10 μM	50 μM	>100 μM
E. coli Δ*tolC* [*fabI*]	0.025 μM	10 μM	50 μM	>100 μM

Figure Captions

Figure 1. Chemical structures of jaceosidin (**1**), jaceidin (**2**), and chrysoplenol B (**3**).

Figure 2. An enzyme inhibition assay was performed to measure the ability of jaceosidin (**1)** to inhibit FabI. The relative enzyme activity was expressed as a percentage of the negative vehicle control ($N = 10$). The relative activity of FabI was determined from the rate of the enzymatic reduction of butenoyl-CoA. Treatment with **1** at a concentration of 30 μM ($N = 4$) did not significantly reduce FabI activity but treatment with **1** at 50 μM ($N = 6$) significantly reduced FabI activity while treatment at 100 μM ($N = 3$) eliminated all observed enzyme activity (*P* values shown from Student's *t*-test comparing each treatment to control).