ALLELOCHEMICAL PHENOLIC ACIDS FROM *GYPSOPHILA PANICULATA*

Shen-Chieh Chou[1], Molly C. Everngam* [2], and John J. Beck† [3]

Department of Chemistry, Sweet Briar College, Sweet Briar, VA 24595.
[1] School of Pharmacy, University of Colorado Health Sciences Center, Denver, CO 80262.
[2] Wildlife International, Ltd, Easton, MD 21601.
[3] USDA-ARS WRRC Plant Mycotoxin Research, 800 Buchanan St., Albany, CA 94710, jbeck@pw.usda.gov.

Abstract

Bioassay-guided fractionation of *Gypsophila paniculata L.* (Caryophyllaceae) resulted in the isolation of the phenolics *p*-coumaric acid [1], dihydroferulic acid [2], and syringic acid [3]. In addition to their noted weak antimicrobial activity, compounds [1] and [3] are known to be potent exuded allelochemicals. Compound [2] has been reported to undergo microbial degradation to vanillic acid [4], which is also known to be an exuded allelochemical. This is the first report of these phenolics from *G. paniculata*.

Keywords: Antimicrobial, Allelochemicals, *Gypsophila paniculata*, Phenolics

Introduction

The genus *Gypsophila* (Caryophyllaceae) is composed of twelve species, of which nine have been introduced into the United States, and two are listed as excluded. The genus is widely used as an ornamental (1). Distributions of the introduced species are primarily restricted to the Northern areas of the United States, with several of the species limited to just one or three states. *G. paniculata* L., commonly known as baby's breath, is the most widely distributed United States species and is listed as a noxious weed in the states of CA and WA (2). Reports on the use of *G. paniculata* as a medicinal plant are few and its use for specific ailments is not mentioned (3). A recent investigation reported the aerial parts of *G. paniculata* as possessing insecticidal activity against the milkweed bug, *Oncopeltus fasciatus* (4). A search of the phytochemical literature reveals triterpenoid saponins to be present in *G. paniculata* (5,6). Saponins are known to possess a wide range of pharmacological, medicinal, antimicrobial, and insecticidal properties (7). Additionally, saponins released from *G. paniculata* have been implicated as controlling rhizosphere bacteria (8). Volatile analysis of *G. paniculata* detected methylbutyric acid, a compound responsible for the unpleasant odor of the inflorescences (9). Herein, we describe the isolation of three phenolic acids from the extracts of dried plant parts of *G. paniculata*, *p*-coumaric acid [**1**], dihydroferulic acid [**2**], and syringic acid [**3**]. Compounds **[1]** and **[3]** are known to be potent exuded allelochemicals (10,11). Compound **[2]** has been reported to undergo microbial degradation to vanillic acid [**4**], which is also known to be an exuded allelochemical (10,12). The weak antibacterial activity of the isolated compounds against *Bacillus subtilis*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* will be discussed. Compound **[2]** was found to be weakly active against the fungi *Candida kefyr* and *Aspergillus niger*.

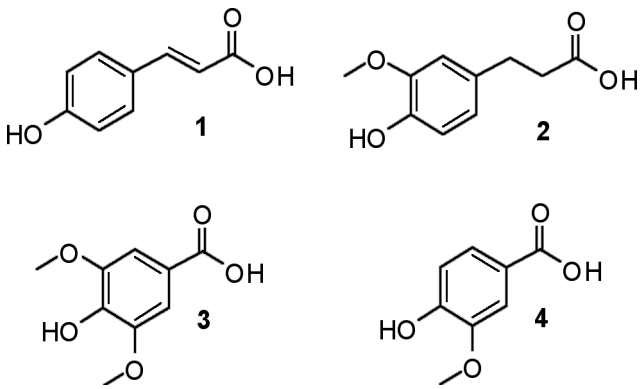

Figure 1. Phenolic acids isolated from G. paniculata (compounds [1-3]), and compound [4], the microbial degradation product of compound [2].

Experimental

Reagent grade solvents used for extractions were purchased from VWR Scientific and distilled prior to use. HPLC grade solvents were purchased from VWR Scientific and used without further purification. Sephadex LH-20 was purchased from Amersham Biosciences Corporation. Microbes were purchased

from Carolina Biological Supply Company. Ampicillin and penicillin were purchased from Wards. For structure verification, compounds **[1]** and **[3]** were purchased from Aldrich Chemical Company. NMR spectra were obtained on a JEOL ECX 400 MHz spectrometer. Purifications via HPLC were performed on a Waters 1525 Binary Pump using a 2487 Dual Wavelength Absorbance Detector, and a YMC-Pack Pro C18 (250 x 10 mm, 5 μm, 12 nm) column for reversed phase or a YMC-Pack Diol-120-NP (250 x 10 mm, 5 μm, 12 nm) column for normal phase. The plant materials were collected from Michigan in September 1971 and supplied by the Medicinal Plant Resources Laboratory, USDA, Beltsville, MD where voucher specimens were preserved (PR 21725).

Nutrient agar and Saboraud dextrose agar were prepared as per the Kirby-Bauer agar disk diffusion method (13) for the growth of the microbial cultures, and performed as previously reported (14). Briefly, assay disks (6 mm) were impregnated with 500 μg of each isolate or fraction and placed on agar plates inoculated with the test microbe. The plates were observed for zones of inhibition, measured as the diameter in mm, after 18 h of incubation at 34ºC. Controls using 6 mm assay disks impregnated with the solvent used and ampicillin, penicillin, or ketoconazole (10 μg, 10 units, or 500 μg, respectively) were utilized with each agar plate. Solvents were removed from the disks *in vacuo* prior to placement on the agar plates. All tests were performed in triplicate and the antimicrobial activity was reported as the average of the zones of inhibition (in mm) produced by the isolates and controls. Inhibitions less than 6.0 mm were reported as a 0.0 mm zone of inhibition.

Table 1. Antimicrobial[a] activity for the isolated phenolics at 500 μg.[b]

	p-coumaric acid	dihydro-ferulic acid	syringic acid	Control
B. subtilis	**19.3**	**20.7**	**19.7**	**31.0**
S. aureus	**16.7**	**10.7**	**17.0**	**37.0**
K. pneumoniae	**12.7**	**0.0**	**0.0**	**17.3**
C. kefyr	**n/a**	**7.3**	**n/a**	**29.0**
A. niger	**n/a**	**7.0**	**n/a**	**19.7**

[a] Microbes tested (and corresponding controls) were the bacteria *Bacillus subtilis* (penicillin), *Staphylococcus aureus* (penicillin), and *Klebsiella pneumoniae* (ampicillin); and the fungi *Candida kefyr* (ketoconazole) and *Aspergillus niger* (ketoconazole). [b] Values provided are the zones of inhibition and are means of triplicate values (mm).

For plant extraction and isolation of compounds, the dried, ground roots, stems, leaves, and flowers (1.58 kg) were subjected to extraction in ethyl acetate (EtOAc) (3 x 24 h) to yield 10.3 g crude extract (0.6% dry weight), followed by hexane (3 x 24 h) to yield 43.6 g of crude extract (2.7% dry weight), and finally methanol (MeOH) (3 x 24 h) to yield 1.4 g of crude extract (0.1% dry weight). Standard antimicrobial bioassay-guided fractionation (14) determined the EtOAc layer warranted further investigation. An aliquot of the EtOAc layer (4.5 g) was chromatographed on Sephadex LH-20 (hexane: CH_2Cl_2: CH_3OH, 3:5:1) to provide four active fractions. One fraction was further separated by semi-preparative HPLC with RP C-18 column (MeOH:H_2O, 1:1, 0-33 min; 3:2 33-56 min; and 1:0 56-70 min) to afford two pure phenolic acids: syringic acid, **[3]** (7.6 mg) and dihydroferulic acid, **[2]** (10.7 mg). A second fraction was further separated with C-18 column chromatography (MeOH:H_2O, 3:7 to 1:0 gradient in 100 mL portions) and the active fraction separated by semi-preparative HPLC with RP C-18 column (MeOH:H_2O, 2:3) to afford *p*-coumaric acid, **[1]** (11.5 mg).

Compounds **[1-3]** were characterized by one- and two-dimensional NMR analyses and were in agreement with spectroscopic data for literature values or commercially obtained samples. Compounds **[1]** and **[3]** were confirmed by comparison with authentic samples, and compound **[2]** was in agreement with previously reported data (15).

Results and Discussion

Table 1 provides the zones of inhibition (in mm) of the isolates against the various microbes used. The Gram-positive bacteria *B. subtilis* and *S. aureus* were the most susceptible to all three phenols with weak- to modest inhibitions of approximately 10-20 mm, with compound **[2]** providing the weakest activity against *S. aureus*. These results are in concurrence with literature reports regarding the Gram-positive antibacterial activity of these phenolics (15-17). Only compound **[1]** provided weak inhibition against the Gram-negative bacterium *K. pneumoniae*. A literature search for corresponding reports did not provide other instances of this finding, although one investigation tested the activity of a plant extract that contained *p*-

coumaric acid as one of the many components and reported no inhibition for this mixture against *K. pneumoniae* (18).

The antifungal activity of compound **[2]** was investigated against the two fungi *A. niger* and *C. kefyr*, and was found to provide only weak inhibition. This supported an earlier report of its activity against *A. flavus* (15). None of the compounds tested provided activity equal to, or greater than the antimicrobial controls.

The extraction of *G. paniculata* afforded three phenolic acids with varying, albeit weak, activity against the tested microbes. Two isolates, compounds **[1]** and **[3]**, are effective allelochemicals, and the third isolate, compound **[2]**, is a precursor to the allelochemical compound **[4]**. This information, combined with the previously discussed report of saponins isolated from *G. paniculata* providing allelopathic affects, offer a compelling argument for the continued investigation of *G. paniculata* as a source of natural antimicrobials for rhizospheres.

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