A New Glucosyl Feruloyl Quinic Acid as a Potential Marker for Roots and Rhizomes of Goldenseal, *Hydrastis canadensis*

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Received April 13, 2004

A new compound, 5-*O*-(4′-[β-D-glucopyranosyl]-*trans*-feruloyl)quinic acid (GPFQ, **10**), is reported from the medicinal plant goldenseal (*Hydrastis canadensis*). A new HPLC method is described and used to show that GPFQ is a potential marker for goldenseal roots (1.0% w/w) and rhizomes (2.3%). GPFQ was found at much lower levels in stems (<0.1%) and could not be detected in leaves. Neochlorogenic acid (**9**), which has not previously been reported from goldenseal, and chlorogenic acid (**6**) reached their highest levels in leaves (0.9% **9** and 0.5% **6**). The main alkaloids, hydrastine (**1**) and berberine (**2**), were highest in rhizomes (2.8% **1** and 4.6% **2**), but palmatine (**5**) was not found in genuine goldenseal.

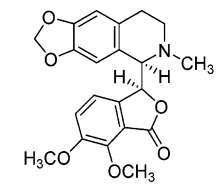
Goldenseal, *Hydrastis canadensis* L. (Ranunculaceae), is a valuable medicinal herb used primarily for its antimicrobial properties.[1−5] It is native to eastern North America, where it is now endangered in the wild due to overharvesting and loss of habitat through deforestation.[6] We are investigating the cultivation of goldenseal in New Zealand and required an analytical method to test the quality of plant material grown here.

The American Herbal Pharmacopoeia states that goldenseal root consists of the fresh or dried roots and rhizomes (underground stems) of *H. canadensis* containing not less than 2.0% hydrastine (**1**) and 2.5% berberine (**2**) (on a dry weight basis).[5] Other alkaloids mentioned in this AHP monograph are hydrastinine (**3**), canadine (**4**), and palmatine (**5**).[5] However, palmatine (**5**) was not found in the comprehensive studies of goldenseal alkaloids by Messana et al.[7] and Chadwick et al.[8] Hydrastine (**1**) and canadine (**4**) are unique to goldenseal, so alkaloid composition has been used to detect adulteration of goldenseal with other, less expensive, berberine-containing plants.[5,9] Other compounds reported from goldenseal include the quinic acid derivative chlorogenic acid (**6**) (common in green plants[10]); two unique quinic acid butyl esters, **7** and **8**;[11] β-sitosterol 3-*O*-β-D-glucoside,[12] and two new *C*-methyl flavonoids.[12]

When we started this project the only reported quantitative methods for analyzing hydrastine and berberine levels in goldenseal were spectrophotometric.[13−15] Subsequently, several HPLC methods have been reported for determining alkaloid levels in goldenseal.[5,9,16−22] We now report an HPLC method that quantifies the main alkaloids, plus three quinic acid derivatives: chlorogenic acid (**6**); neochlorogenic acid (**9**), which has not previously been reported from goldenseal; and a new compound, 5-*O*-(4′-[β-D-glucopyranosyl]-*trans*-feruloyl)quinic acid, GPFQ (**10**). Analyses are presented for five different parts of goldenseal plants.

Results and Discussion

We developed a mobile phase and acid modifier combination (methanol/water with 0.1% formic acid) that gave a maximum number of resolved peaks in reversed-phase HPLC analyses of goldenseal extracts (Figure 1). Peaks for berberine (**2**), hydrastine (**1**), hydrastinine (**3**), and chlorogenic acid (**6**) were identified by matching retention times and UV-DAD spectra with commercial standards. A peak coeluting with palmatine (**5**) was found in a commercial sample but not in any of the goldenseal grown for this project. Canadine (**4**) was synthesized by reduction of berberine (**2**),[23] and the HPLC peak in goldenseal extracts identified by its UV-DAD spectrum and co-injection.



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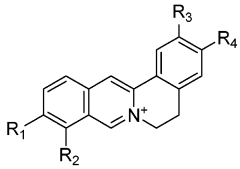
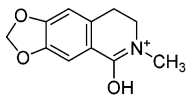


2 $R_1 = R_2 = CH_3O$, $R_3 + R_4 = O\text{-}CH_2\text{-}O$

5 $R_1 = R_2 = R_3 = R_4 = CH_3O$



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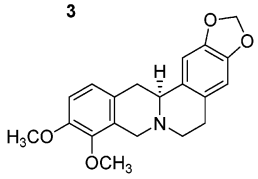

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Two large unidentified peaks were observed during early HPLC analyses of goldenseal. One peak, which was particularly prominent in leaf extracts (Figure 1), had the same UV spectrum as chlorogenic acid, so it was expected to be a caffeate. Isolation under normal laboratory conditions gave both *E*- and *Z*-caffeate isomers, which we interpreted as due to photoisomerization, observed previ-

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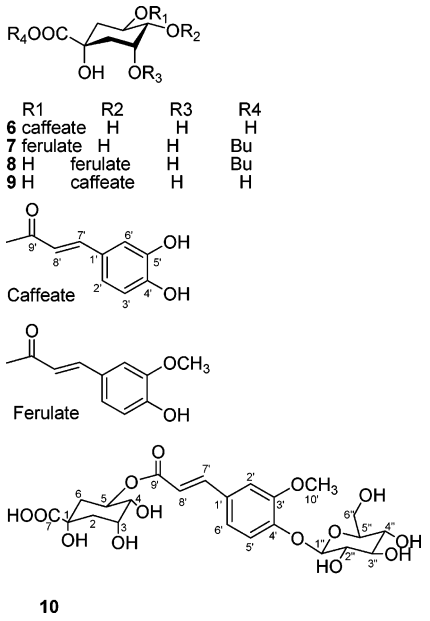
10.1021/np049868j CCC: $27.50
Published on Web 10/15/2004



ously for another caffeate.[24] Isolation with minimal exposure to light gave an almost pure *E*-caffeate. This was identified as neochlorogenic acid (**9**) by comparison with published NMR data.[25] Neochlorogenic acid (**9**) has not been previously reported in goldenseal, but it is often found together with chlorogenic acid (**6**).[26]

The largest unidentified peak in root extracts (labeled GPFQ, Figure 1) showed a UV spectrum similar to the spectra of chlorogenic acid (**6**) and neochlorogenic acid (**9**) but with absorption maxima offset to shorter wavelengths. GPFQ was isolated from goldenseal rhizomes by extraction with methanol followed by water/chloroform partitioning. The water-soluble compounds were fractionated on a C_{18} reversed-phase bench column followed by C_{18} HPLC to give the pure compound. Electrospray-ionization MS showed a strong $[M - H]^-$ ion at 529 Da, and HRFABMS gave a $[M + H]^+$ ion appropriate for the molecular formula $C_{23}H_{30}O_{14}$.

The structure of GPFQ (**10**) was identified using 2D NMR data (Table 1), which showed a quinic acid unit acylated at C-5. This was confirmed by very similar ^1H and ^{13}C NMR chemical shifts to chlorogenic acid (**6**) (Table 1) and similar $^1H-^1H$ coupling constants.[27] The acyl group was shown to be ferulate, rather than caffeate as in chlorogenic acid (**6**), by key CIGAR and NOESY correlations (Table 1). In particular, an aromatic methoxyl ^1H NMR signal at 3.89 ppm showed both a $^4J_{C-H}$ HMBC correlation and a NOESY correlation to an aromatic methine (^1H 7.25 ppm, ^{13}C 112.5 ppm) assigned as C-2′. This structural assignment required H-5′ and H-6′ to have coincident chemical shifts, and thus no observable *ortho* coupling. Coincident H-5′ and H-6′ ^1H NMR signals have been reported for 4′-(β-D-glucopyranosyl)-*trans*-ferulic acid,[28] which showed similar ^1H and ^{13}C chemical shifts for the rest of the ferulate portion of GPFQ. The remaining unassigned signals in our GPFQ (**10**) molecule (1″ to 6″, Table 1) had chemical shifts very similar to the glycoside portion of 4′-(β-D-glucopyranosyl)-*trans*-ferulic acid.[28] The $^1H-^1H$ coupling constants (Table 1) confirmed that GPFQ (**10**) contained a β-glucopyranoside.[29]

The absolute configurations of the β-glucopyranoside and quinic acid units are assumed to be those normally found in plants, giving the proposed structure **10**. A literature search found no matches to this exact structure, but one report of an epimeric compound, 4′-*O*-(α-D-glucopyranosyl)-5-*trans*-feruloyl quinic acid, in a coffee bean extract.[30] The partial ^1H and ^{13}C NMR data reported for this compound[30] were mostly similar to ours, but the anomeric proton signal H-1″ at 5.35 ppm differed from that of GPFQ (**10**) at 4.97 ppm (Table 1).

Using our HPLC method we found certain limitations with the extraction methods in the literature. Repeat sonication of the sample,[18,19] single sonication, or soaking with methanol or ethanol[20,31] did not result in full recovery of berberine (**2**) or hydrastine (**1**). Soxhlet extraction[17] took 24 h per sample, whereas refluxing with methanol (three times 20 min) gave good recovery (<5% more of the major compounds were recovered by a fourth extraction). Our HPLC method is precise for the main analytes, berberine (**2**), hydrastine (**1**), and GPFQ (**10**) (relative standard deviation [RSD] <1%), and reasonably precise for the other analytes (RSD <5%).

This HPLC method was used to analyze goldenseal grown in New Zealand. Three plants were harvested and separated into five component parts: roots, rhizomes, lower stems, upper stems, and leaves. The division between lower

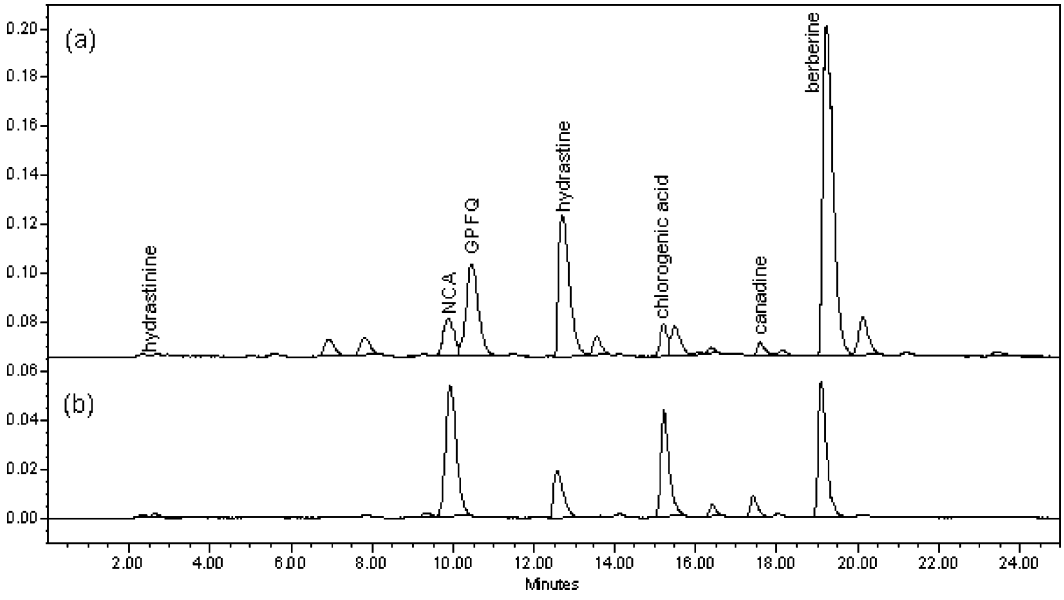


Figure 1. HPLC chromatograms of extracts of goldenseal (a) root and (b) leaf (detection at 295 nm).

Table 1. NMR Data for 5-*O*-(4′-[β-D-Glucopyranosyl]-*trans*-feruloyl)quinic Acid (**10**)[a]

	^{13}C	^{1}H[b]	CIGAR[c]	COSY	NOESY[c]
1	75.4 [76.4]		H-3		
2 ax	38.2 [38.5]	2.17 (dd, 14.0, 3.0) [2.18]	H-3	H-3, H-2	NO
2 eq		2.04 (ddd, 14.0, 5.5, 2.0) [2.05]		H-3, H-2	NO
3 ax	71.3 [71.6]	4.16 (dt, 5.5, 3.0) [4.17]	NO[d]	H-3, H-2	H-4, H-2s
4 ax	73.4 [73.7]	3.73 (dd, 8.0, 3.0) [3.73]	H-3	H-5, H-3	H-5, H-3
5 ax	72.1 [72.5]	5.33 (td, 9.5, 4.5) [5.33]	NO	H-4, H-6s	H-4, H-6s
6 eq	38.8 [39.1]	2.23 (ddd, 12.5, 4.0, 2.0) [2.23]	H-5, H-4	H-5, H-6	NO
6 ax		2.10 (dd, 13.0, 9.5) [2.08]		H-5, H-6	NO
7	177.0 [177.3]		NO		
1′	130.6 [149.9]		H-7′, H-2′, H-5′/6′, H-8′		
2′	112.5 [115.5]	7.25 (br s) [7.05]	H-7′, H-5″/6′, **H-10′**[e]	H-5′/6′	H-7′, H-8′, **H-10′**
3′	151.0 [128.1]		H-2′, H-5′/6′, **H-10′**		
4′	150.1 [147.1]		H-2′, H-5′/6′, **H-1″**		
5′	117.5 [116.8]	7.17 (m) [6.78]	NO	H-2′[c]	H-7′, H-8′, H-1″[f]
6′	123.5 [123.3]	7.17 (m) [6.95]	H-7′, H-2′, H-5′	H-2′[c]	H-7′, H-8′, H-1″[f]
7′	146.2 [147.4]	7.63 (d, 16.0) [7.56]	H-2′, H-6′, H-8′	H-8′	H-2′, H-6′
8′	117.4 [115.5]	6.43 (d, 16.0) [6.26]	H-7′	H-7′	H-2′, H-6′
9′	168.2 [168.9]		H-7′, H-8′, **H-5**		
10′	56.8	3.89 (s)	NO		H-2′
1″	102.2	4.97 (d, 7.5)	H-3″, H-2″, H-5″	H2″	H-5′, H-5″
2″	77.8[f]	3.47 (t, 8.0)	H-1″, H-3″, H-4″	H1″	NR[g]
3″	74.8[f]	3.5 (dd, 8.0, 9.0)	H-2″	NR	H-1″, H-4″
4″	71.3	3.39 (dd, 9.0, 8.0)	H-6″	NR	NR[g]
5″	78.3	3.43 (ddd, 9.0, 5.5, 2.5)	H-1″, H-6″, H-3″, H-4″	H-6″	H-1″
6″	62.5	3.68 (dd, 12.0, 5.0)	H-5″	H-6″, H-5″	H6″, H5″
		3.87 (dd, 12.0, 2.0)		H-6″	H6″, H5″

[a] In CD_3OD, shifts in ppm [corresponding chlorogenic acid (**6**) signals]. [b] (Multiplicity, *J* in Hz). [c] Key correlations in **bold**. [d] None observed. [e] Four-bond correlation. [f] Correlations from overlapping signals. [g] Not resolved.

Table 2. HPLC Analyses of Goldenseal Samples[a]

plant part	hydrastinine (**3**)	NCA (**9**)	GPFQ (**10**)	hydrastine (**1**)	CA (**6**)	canadine (**4**)	berberine (**2**)	palmatine (**5**)
root[b]	0.080	0.19	1.10	1.90	0.32	0.26	3.78	NF[c]
rhizome[b]	NF	0.23	2.26	2.77	0.17	0.20	4.62	NF
lower stem[b]	0.018	0.12	0.08	0.43	0.24	0.26	1.83	NF
upper stem[b]	0.010	0.10	0.01	0.31	0.20	0.07	1.25	NF
leaf[b]	0.030	0.90	NF	1.01	0.51	0.43	1.50	NF
LSD[d]	0.013	0.15	0.34	0.21	0.19	0.08	0.55	NF
root[e]	NF	0.02	0.18	0.31	0.03	NF	0.72	0.05

[a] Levels are % w/w. [b] Means of three samples. [c] Not found. [d] Least significant differences ($P < 0.05$, df = 8) between values above. [e] Single commercial sample.

stems and upper stems was about 50 mm from the ground to simulate automated harvesting of the leaves. The analyses showed large, statistically significant ($p < 0.05$), differences in composition between different plant parts (Table 2). GPFQ (**10**) is a major component in goldenseal roots and rhizomes along with hydrastine (**1**) and berberine (**2**) (Table 2). Therefore, it is surprising that this compound has not been noted in previous analyses.[1−5,9] GPFQ is not peculiar to our New Zealand-grown goldenseal, as we detected this compound in a commercial sample of goldenseal (Table 2). We found similar levels of GPFQ (**10**) in goldenseal grown on the South Island of New Zealand (Crop & Food Research, unpublished results).

The compounds most similar to **10** previously reported from goldenseal are the feruloyl quinic acid butyl esters **7** and **8** found by Gentry et al.[11] When we treated goldenseal roots by their isolation method,[11] our HPLC analysis of the cold ethanol extract showed the usual alkaloid profile plus chlorogenic acid (**6**), neochlorogenic acid (**9**), and GPFQ (**10**). However, after fractionating the dried extract between chloroform and aqueous acid, we could not detect compound **6**, **9**, or **10** in either fraction. Therefore, any isolation method involving acid treatment of goldenseal would probably not detect the quinic acid derivatives **6**, **9**, and **10**.

Other researchers who used simple solvent extraction do not note quinic acid derivatives or unknown peaks.[5,9] We found that goldenseal root and rhizome extracts prepared by our method and by the American Herbal Pharmacopoeia (AHP) method[5] both gave the same HPLC profile when analyzed using our HPLC method. When the two extracts were analyzed using the AHP HPLC method,[5] peaks for berberine, hydrastinine, hydrastine, and canadine were identified by their standard retention times. Chlorogenic acid (**6**), neochlorogenic acid (**9**), and GPFQ (**10**) eluted with the solvent front, which may explain why no other researchers have reported neochlorogenic acid and GPFQ in goldenseal.

The AHP[5] and Weber et al.[9] suggest that the presence or absence of various alkaloids could indicate adulteration of goldenseal samples with cheaper, berberine-containing plants. Palmatine (**5**) was not detectable in any of our pure goldenseal samples, but it was present in the commercial sample that we analyzed (Table 2). We suggest that GPFQ (**10**) has potential as a unique (i.e., not currently reported from any other source) marker for goldenseal roots and rhizomes. This compound should be readily detected using our extraction and HPLC method, and its HPLC peak identified by LC-DAD to show its characteristic UV spectrum and by LC-MS in the negative-ion mode. Furthermore, GPFQ (**10**) was not detectable in our leaf samples and neochlorogenic acid (**9**) levels were significantly higher in leaves (Figure 1 and Table 2). Therefore, leaf extracts might be distinguishable from root/rhizome extracts by

analyses of compounds **9** and **10**, but there are major seasonal changes in their levels (Crop & Food Research, unpublished data).

It seems that GPFQ (**10**) does not contribute to the antimicrobial properties of goldenseal, since it was not active (minimum inhibitory concentration >1 mg/mL) against methicillin-resistant *Staphylococcus aureus*. Berberine (**2**) was antimicrobial (MIC 0.06 mg/mL) in this assay, as expected.[11,32,33] However, chlorogenic acid (**6**) and related quinic acid derivatives do have a range of biological activities,[34] so the new compound, GPFQ (**10**), may contribute to the overall biological effect of goldenseal.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. LR MS, HR MS, UV, and IR spectra were recorded on Shimadzu LC-MS QP8000α, VG70-250S double focusing magnetic sector mass spectrometer, JASCO V-550, and Perkin-Elmer 1600 FTIR instruments, respectively. NMR spectra, at 25 °C, were recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C on a Varian INOVA-500 spectrometer. Chemical shifts are given in ppm on the δ scale referenced to the solvent peaks C*H*D$_2$OD at 3.30 and *C*D$_3$OD at 49.0 ppm.

Plant Material. Goldenseal (*Hydrastis canadensis* L.) was planted at Ruakura, New Zealand, in October 1996 and harvested in December 2001. Plants were sourced from North America in 1990 and quarantined at Ruakura. A voucher specimen has been kept at Invermay (number 820). Commercial goldenseal capsules were purchased in Dunedin.

Neochlorogenic Acid (9). The isolation of neochlorogenic acid (**9**) was carried out with minimal exposure to light. Goldenseal leaf (15.6 g) was refluxed with MeOH (3 × 300 mL, 40 min) and filtered, and the solvent evaporated to give a green solid (5.74 g). A portion of this solid (5.2 g) was partitioned between H_2O and $CHCl_3$. A portion of the H_2O fraction (0.3 g out of 3.74 g) was purified by semipreparative HPLC: Phenomenex Luna C_{18} column (250 × 10 mm), with a flow rate of 5 mL/min and detection at 295 nm. The mobile phase was methanol in 0.1% formic acid aqueous: t_0 = 25%, t_{10} = 50%, t_{11} = 25%, $t_{12.5}$ = 25%. Neochlorogenic acid (**9**) (17 mg) was collected from 5 to 6 min and identified by comparing NMR data with published values.[25]

5-*O*-(4′-[β-D-Glucopyranosyl]-*trans*-feruloyl)quinic acid (10). The isolation of GPFQ (**10**) was carried out with minimal exposure to light. Ground goldenseal rhizome (5.07 g) was refluxed with MeOH (200 mL, 60 min) and filtered, and the solvent was evaporated to give an orange solid (1.21 g). This was partitioned between H_2O and $CHCl_3$. A portion of the H_2O fraction (815 mg out of 1.01 g) was applied to a C_{18} column (Waters Sep-pak Vac 35 cm^3, tC_{18} cartridge) and eluted with increasing proportions of MeOH in water. GPFQ (**10**) was found in fractions 16−18 (20−50% MeOH). Fraction 18 (244 mg) was purified by semipreparative HPLC: Phenomenex Luna C_{18} column (250 × 10 mm), with a flow rate of 5 mL/min, gradient conditions as for the analytical method, and detection at 295 nm. GPFQ (**10**) (13 mg) was collected from 10 to 11 min 30 s.

GPFQ (10): off-white powder; $[\alpha]_D^{19}$ −53° (*c* 0.02, MeOH), $[\alpha]_D^{16}$ −58° (*c* 0.02, H_2O); UV (MeOH) λ_{max} (log ϵ) 217 (4.32), 232 (4.31), 292 (4.21), 320 (4.28) nm; IR (KBr disk) ν_{max} 3800−2400 (OH), 2930 (CH), 1700 (C=C−CO−OR), 1635 (COOH), 1510, 1260, 1190, 1130, 1070 (C=C, trans) 1041, 808 (aromatic CH) cm^{-1}; NMR data in Table 1; ESIMS *m/z* 529 $[M - H]^-$; HRFABMS *m/z* 531.1691 (calcd for $C_{23}H_{31}O_{14}$, 531.1714).

Analytical Standards. A stock solution of 500 μg/mL berberine chloride dihydrate (Sigma) in MeOH was prepared by sonicating for 2 min (this solution was stable at −20 °C for 6 months, as determined by HPLC comparison with a freshly prepared solution). Standard solutions of hydrastine (Sigma), hydrastinine chloride 0.5 hydrate (Sigma), chlorogenic acid (Aldrich), palmatine 1.5 mol methanol (Aldrich), neochlorogenic acid, GPFQ, and canadine were prepared in MeOH for measurement of the relative response factors (Table 3). These factors were used to calculate the level of each analyte as shown in the structural formulas **1**−**6**, **9**, and **10**, i.e., without including any counterions or solvate molecules present in reference samples or the extracts.

Table 3. HPLC Retention Times and Relative Responses for Goldenseal Compounds

compound	retention (min)	relative response
hydrastinine (**3**)	2.6	0.82
NCA (**9**)	9.5	0.52
GPFQ (**10**)	10.2	1.40
hydrastine (**1**)	12.2	1.56
CA (**6**)	14.7	0.52
canadine (**4**)	17.0	2.30
berberine (**2**)	18.6	1.00

Plant Extraction. Finely ground plant material (1 g) was refluxed with MeOH (40 mL, 20 min), and the solution was cooled to room temperature and filtered through cotton wool. The plant material and cotton wool were refluxed twice more with MeOH (20 mL, 20 min), followed by cooling and filtering. The combined extracts were made up to 100 mL with MeOH. An aliquot of extract was filtered through a PTFE filter (0.45 μm) for HPLC analysis. Extracts were stored in the dark at −20 °C prior to HPLC analysis and were stable for at least two weeks if unopened.

HPLC Analyses. Analyses were carried out at 25 °C on a C_{18} column (Phenomenex Prodigy ODS(3) 5 μm 100 Å, 250 × 4.6 mm) with a 2 × 4 mm C_{18} guard column. Peaks were detected at 295 nm for the standard analysis and also monitored at 235 nm. A gradient program was used: the initial solvent mix was 20−80 MeOH−0.1% formic acid in H_2O held for 3 min, changing linearly to 35:65 at 10 min, held for 7 min, then back to 20:80 at 18 min with a 7 min hold for equilibration prior to the next analysis. The flow rate was 1.0 mL/min, with an injection volume of 5 μL. The HPLC was controlled by Millennium32 software (version 3.05, 1998, Waters Corporation). The HPLC component system was a 717 auto sampler, 600 controller, and a 2487 programmable multiwavelength detector. The composition of the analytes was calculated using Millennium 32, with a one-point calibration (berberine, duplicate preparation, intercept through zero) and repeated standard injections to ensure stability with time. Retention times and response factors are listed in Table 3.

The calculation for the composition of the plant material is

$$\text{composition (\%)} = \frac{A_{\text{sample}} \times V_{\text{sample}} \times \text{RRF} \times 100\%}{\text{RF}_{\text{std}} \times W_{\text{sample}} \times 1000\ \mu\text{g/mg}}$$

$$\text{RRF} = \frac{\text{RF}_{\text{std}}}{\text{RF}_{\text{analyte}}}$$

A_{sample} is the peak area of the component in the sample solution (area counts), RF_{std} is the mean response factor of berberine in the standard solutions (response factor = [(area/conc (μg/mL))], V_{sample} is the volume of the sample solution (mL), and W_{sample} is the weight of sample taken (mg). RRF is the relative response factor of the component relative to berberine. Therefore, the sample weight should be entered in mg, the dilution 10, and the standard concentrations as μg/mL to produce a % w/w result in Millenium32.

Antimicrobial Assay. The *Staphylococcus aureus* strain used was 1126, methicillin resistant. The bacterial culture was maintained on tryptic soy agar; it was subcultured every two weeks and stored at 4 °C. A single colony was used to inoculate 10 mL of Todd Hewitt broth (THB). The broth was then incubated at 37 °C for 18 h, then the bacterial culture was diluted to an $OD_{600\ nm}$ 0.01 using THB. The test compounds were serially diluted (2-fold) in THB and 100 μL volumes dispensed into the wells of a flat-bottom 96-well microtiter plate. Aliquots (100 μL) of the diluted overnight bacterial culture were added to each well. All tests were conducted in triplicate and controls included. The microtiter plate was

incubated at 37 °C for 24 h in a plate reader (Multiskan ascent microtiterplate reader) with absorbance readings (595 nm) taken every 2 h.

Acknowledgment. We thank M. Thomas and I. Stewart for assistance with NMR spectra, L. Larsen for help with determining the structure, R. Butler for the statistical analysis, P. Bremer and M. Dufour for antimicrobial assays, and I. Stewart and A. Matich for mass spectrometry analysis. This research was funded by the New Zealand Foundation for Research, Science and Technology, contract C02X0211.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compound **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP049868J