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Pyrano-isoflavans from *Glycyrrhiza uralensis* with Antibacterial Activity against *Streptococcus mutans* and *Porphyromonas gingivalis*

Jacquelyn R. Villinski,[†] Chantal Bergeron,[†] Joseph C. Cannistra,[‡] James B. Gloer,[‡] Christina M. Coleman,[§] Daneel Ferreira,[§] Jabrane Azelmat,[⊥] Daniel Grenier,[⊥] and Stefan Gafner*[,†,∥]

[†]Tom's of Maine, 302 Lafayette Center, Kennebunk, Maine 04043, United States

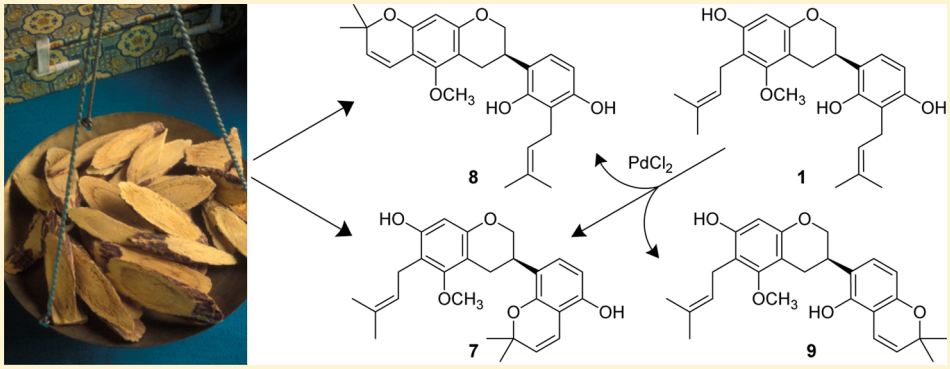
[‡]Department of Chemistry, University of Iowa, Iowa City, Iowa 52242, United States

[§]Department of Pharmacognosy and the Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, Mississippi 38677, United States

[⊥]Groupe de Recherche en Écologie Buccale, Faculté de Médecine Dentaire, Université Laval, Quebec City, Quebec G1V 0A6, Canada

[∥]American Botanical Council, Austin, Texas 78714, United States

Supporting Information



ABSTRACT: Continuing investigation of fractions from a supercritical fluid extract of Chinese licorice (*Glycyrrhiza uralensis*) roots has led to the isolation of 12 phenolic compounds, of which seven were described previously from this extract. In addition to these seven metabolites, four known components, 1-methoxyerythrabyssin II (**4**), 6,8-diprenylgenistein, gancaonin G (**5**), and isoglycyrol (**6**), and one new isoflavan, licorisoflavan C (**7**), were characterized from this material for the first time. Treatment of licoricidin (**1**) with palladium chloride afforded larger amounts of **7** and also yielded two new isoflavans, licorisoflavan D (**8**), which was subsequently detected in the licorice extract, and licorisoflavan E (**9**). Compounds **1**−**9** were evaluated for their antibacterial activities against the cariogenic *Streptococcus mutans* and the periodontopathogenic *Porphyromonas gingivalis*. Licoricidin (**1**), licorisoflavan A (**2**), and **7**−**9** showed antibacterial activity against *P. gingivalis* (MICs of 1.56−12.5 μg/mL). The most potent activity against *S. mutans* was obtained with **7** (MIC of 6.25 μg/mL), followed by **1** and **9** (MIC of 12.5 μg/mL). This study provides further evidence for the therapeutic potential of licorice extracts for the treatment and prevention of oral infections.

Chinese licorice (*Glycyrrhiza uralensis* Fisch., Fabaceae) is the main licorice species used in Asia and is called "gan-cao" in Chinese, which means "sweet herb". The name is due to the presence of glycyrrhizin, a triterpene diglucuronate that gives the root its typical sweet taste. Licorice has a long history of use. The first recorded use was as a drug for the treatment of wounds as described in the book *Wu Shi Er Bing Fang*, dating to the second century B.C. According to Liu and Liu,[1] the roots and rhizomes of *G. uralensis* are used in traditional Chinese medicine to strengthen the spleen and augment "Qi" (energy), to moisten the lungs and stop cough, and to soothe spasms and diminish pain. Licorice is used in as many as half of all traditional Chinese medicine prescriptions to enhance the effectiveness of other ingredients, reduce toxicity, or improve taste.[2]

Dental caries and periodontal diseases are oral infections that affect a large proportion of the population throughout the world. In an earlier study, the antibacterial activities of the main isoflavonoids and coumarins of *G. uralensis* were evaluated against major oral pathogens.[3] As a continuation of this work to characterize the metabolites present in the supercritical fluid extract, the antibacterial activities of several minor compounds

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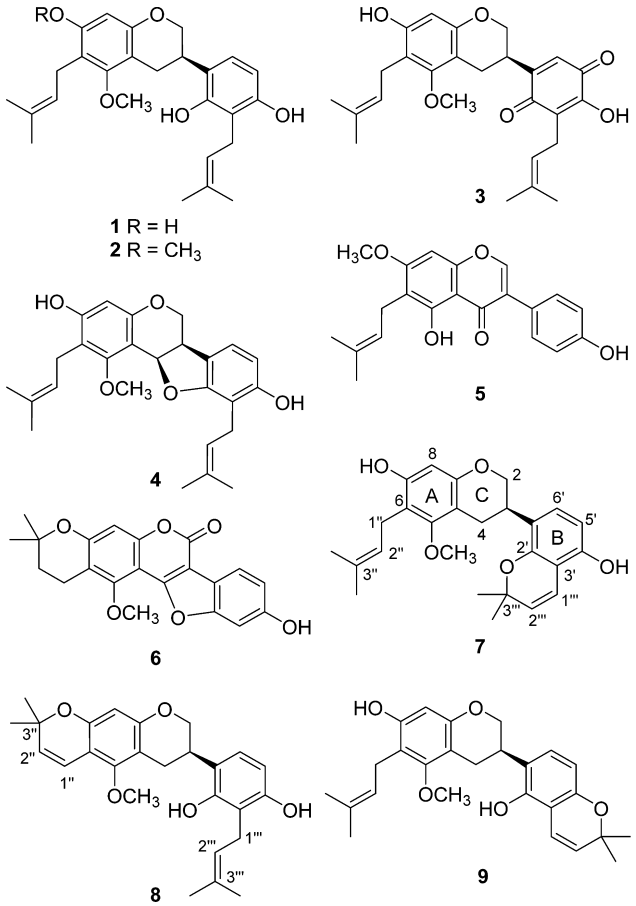
were assessed against the cariogenic *Streptococcus mutans* and the periodontopathogenic *Porphyromonas gingivalis*. *S. mutans* is considered the principal etiological agent of dental caries formation through its aciduric, acidogenic, and adhesion properties.[4] *P. gingivalis* has been identified as a key pathogen contributing to chronic periodontitis,[5] as the establishment of *P. gingivalis* in subgingival sites induces an inflammatory response that leads to gingival tissue destruction and progressive loss of alveolar bone around the teeth.[6]



RESULTS AND DISCUSSION

Additional separation of fractions obtained earlier from a supercritical fluid extract of *G. uralensis* roots led to the isolation of the known licoricidin (**1**),[7] licorisoflavan A (**2**),[8] glyasperin D,[9] licoricone,[10] 1-methoxyficifolinol,[11] glycyrin,[9] and licoriquinone B (**3**),[3] along with four additional known compounds, 1-methoxyerythrabyssin II (**4**),[12,13] 6,8-diprenylgenistein,[14] gancaonin G (**5**),[10] and isoglycyrol (**6**),[15] isolated for the first time from this source. The structures of the known compounds and the new isoflavan, licorisoflavan C (**7**), were determined based on comparison of spectroscopic and spectrometric information with published data.

The molecular formula of **7**, $C_{26}H_{30}O_5$, was determined by HRESITOFMS, which showed an $[M + H]^+$ ion at m/z 423.2178, calcd for $[C_{26}H_{30}O_5 + H]^+$, m/z 423.2172. The presence of an isoflavan skeleton was deduced from the NMR data. In the ^{1}H NMR spectrum, the signals corresponding to H-2α, H-2β, H-3, H-4α, and H-4β (Table 1) were similar to the signals of the C-ring protons present in the ^{1}H NMR spectra of known isoflavans isolated from the extract. In addition, the spectrum contained signals for three aromatic protons, of which two were *ortho*-coupled, one methoxy group, one prenyl group, and a 2,2-dimethyl-2*H*-pyran unit.

Owing to the small amount of material isolated, only partial ^{13}C NMR data could be obtained. In the HSQC data, the aromatic proton resonances at δ 6.88, 6.40, and 6.17 correlated with the ^{13}C NMR resonances at δ 128.0, 108.4, and 99.9, respectively, consistent with the pattern observed for previously isolated licorice isoflavans, e.g., **1** and **2**, with protons at C-5′, C-6′, and C-8 and oxygen substituents at C-5, C-7, C-9, C-2′, and C-4′. The positions of the prenyl moiety and dimethyl-2*H*-pyran ring could not be established conclusively with the available data. Attempts therefore were made to synthesize the three possible regioisomers, 7,2′-dihydroxy-5-methoxy-6-prenyl-6‴,6‴-dimethylpyrano[2‴,3‴:4′,3′]isoflavan, 7,4′-dihydroxy-5-methoxy-6-prenyl-6‴,6‴-dimethylpyrano[2‴,3‴:2′,3′]-isoflavan, and 2′,4′-dihydroxy-5-methoxy-3′-prenyl-6″,6″-dimethylpyrano[2″,3″:7,6]isoflavan, using licoricidin (**1**) as the starting material. This material was abundantly available to our research group from previous isolation efforts involving *G. uralensis* (see Supporting Information).

A simple one-step process for oxidative cyclization of an isoprenyl moiety to form a pyran ring using 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) was reported by Jain and Sharma in 1974.[16] It was hypothesized that all three possible oxidative cyclization products would be formed during exposure of **1** to these conditions, but the resulting reaction mixture contained only two products, both showing an $[M + H]^+$ ion at m/z 423 by ESIMS. After separation by preparative HPLC, one of the products was identified as the pterocarpan **4**, based on comparison of spectroscopic and spectrometric information with published data.[12,13] The second compound (**8**) was found to be an isomer of **4** based on HRESITOFMS data, which showed an $[M + H]^+$ ion at m/z 423.2191 (calcd 423.2172). The UV spectrum of **8** differed from that of **7**, with maxima at 277, 285, and 306 nm, as opposed to 224, 281, and 310 nm. The ^{1}H NMR spectrum (Table 1) exhibited resonances corresponding to the same subunits found in **7**, but the shifts did not match those observed for this isolate. The most significant differences were observed in the resonances for H-8 and the prenyl moiety. The structure was elucidated ultimately by analysis of the HMBC data. In particular, the correlations between OH-2′ (δ 7.20, br s) and C-1′ (δ 120.4), C-2′ (δ 154.1), and C-3′ (δ 116.3) and between OH-4′ (8.17, br s) and C-3′ were used to confirm the structure as the target regioisomer, 2′,4′-dihydroxy-5-methoxy-3′-prenyl-6″,6″-dimethylpyrano[2″,3″:7,6]isoflavan, licorisoflavan D (**8**). Comparison of the HPLC retention time and UV and MS data with those of a minor component present in one of the licorice fractions obtained during purification led to the conclusion that **8** is a naturally occurring constituent of the investigated licorice root extract.

A second synthetic approach was used to generate the two remaining regioisomers, **7** and **9**, as the use of DDQ did not affect pyran-ring formation onto the B-ring. Initially, **1** was acetylated using Ac_2O–pyridine to prepare a mixture of 7,2′-diacetyl-, 7,4′-diacetyl-, and 2′,4′-diacetyllicoricidin. These products were separated by HPLC, and their structures determined by NMR spectroscopy. 7,2′-Diacetyllicoricidin was subjected to oxidative cyclization[17] with $PdCl_2$. Subsequent deacetylation using 1.2 N NaOH and purification by preparative HPLC afforded the new 7,2′-dihydroxy-5-methoxy-6-prenyl-6‴,6‴-dimethylpyrano[2‴,3‴:4′,3′]isoflavan

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Table 1. ^{1}H NMR Spectroscopic Data for Licorisoflavans C, D, and E (7–9)

	licorisoflavan C (7)[a]	licorisoflavan D (8)		licorisoflavan E (9)	
position	δ_H, mult. (J in Hz)[b]	δ_H, mult. (J in Hz)[c]	HMBC[c]	δ_H, mult. (J in Hz)[c]	HMBC[c]
2α (eq)	4.16, ddd (2.0, 3.4, 10)	4.22, ddd (2.2, 3.4, 10)	3,4,9,10[d],1′	4.17, dt (10, 2.2)	3,4,9,1′
2β (ax)	3.97, t (10)	3.96, t (10)	3,4,9,1′	3.95, br t (10)	3,4,9,1′
3	3.35, m	3.39, dddd (3.4, 5.1, 10, 11)	2,4,10,1′,2′,6′	3.38, m	2,4,10,1′,2′,6′
4α (eq)	2.88, ddd (2.0, 5.3, 16)	2.91, ddd (2.2, 5.1, 16)	2,3,5,8[d],9, 10,OCH$_3$-5[d],1′	2.89, ddd (1.4, 4.5, 16)	2,3,5,9,10,1′
4β (ax)	2.80, dd (11, 16)	2.74, dd (11, 16)	2,3,5,7[d],8[d], 9,10,OCH$_3$-5[d],1′	2.74, dd (11, 16)	2,3,5,6[d],8[d], 9,10,1′
8	6.17, s	6.04, s	4[d],5,6,7,9,10,1″[d]	6.17, s	4[d],5[d],6,7, 9,OCH$_3$-5[d],1″[d]
5′	6.40, d (8.4)	6.46, d (8.4)	3[d],1′,2′[d],3′,4′,1‴[d]	6.34, d (8.3)	1′,2′[d],3′,4′,1‴[d]
6′	6.88, d (8.4)	6.83, d (8.4)	2[d],3,2′,3′[d],4′	6.95, d (8.3)	3,2′,3′[d],4′
1″a	3.30, dd (7.0, 14)	6.52, d (9.9)	5,6,7,8,2″,3″,4″,5″, OCH$_3$-5[d]	3.29, dd (7.2, 14)	5,6,7,2″,3″,5″[d]
1″b	3.25, dd (7.0, 14)			3.23, dd (6.8, 14)	5,6,7,2″,3″,5″[d]
2″	5.25, m	5.58, d (9.9)	5[d],6,7[d],8[d], 3″,4″,5″	5.23, m	6,1″,4″,5″
4″	1.64, br s	1.36, s[g]	1″[d],2″,3″, 5″	1.63, br s	2″,3″,5″
5″	1.75, br s	1.38, s[g]	1″[d],2″,3″,4″	1.74, br s	2″,3″,4″
1‴	6.70, d (10)	3.45, br d (7.0)	2′,3′,4′,2‴,3‴,4‴[d],5‴[d]	6.79, d (10)	2′,3′,4′,2‴,3‴,4‴[c],5‴[d]
2‴	5.66, d (10)	5.26, m	3′,1‴,4‴,5‴	5.70, d (10)	1′[d],3′,4′[d],3‴,4‴,5‴
4‴	1.42, s[e]	1.66, br s	3′[d],2‴,3‴,5‴	1.37, s	2‴,3‴,5‴
5‴	1.43, s[e]	1.77, br s	3′[d],2‴,3‴,4‴	1.37, s	2‴,3‴,4‴
OCH$_3$-5	3.69, s	3.72, s	5	3.67, s	5
OH-7	8.39, br s[f]	-	-	8.37, br s[e,f,g,h]	
OH-2′	-	7.20, br s	1′,2′,3′	8.11, br s[e,f,g,h]	
OH-4′	8.08, br s[f]	8.17, br s	3′	-	

[a]No HMBC experiment was performed on this semisynthetic compound. [b]Data collected using acetone-d_6 at 500 MHz. [c]Data collected using acetone-d_6 at 600 MHz. [d]Indicates a correlation across more than three bonds. [e,f,g,h]These assignments are interchangeable.

(**9**) in low yield (1.5 mg, corresponding to 0.6%). The structure was confirmed by analysis of ^{1}H and ^{13}C NMR data (Tables 1 and 2), and the product named licorisoflavan E (**9**).

The above approach involving acetylation, cyclization, and subsequent ester hydrolysis could not be used effectively for the synthesis of 7,4′-dihydroxy-5-methoxy-6-prenyl-6‴,6‴-dimethylpyrano[2‴,3‴:2′,3′]isoflavan (**7**), due to the initial low yield of 7,4′-diacetyllicoricidin (6.7 mg; 2.2%) and the representative overall low yield of **9**. Subsequent attempts to improve yields were unsuccessful. This prompted the use of a third synthetic approach employing $PdCl_2$ to effect oxidative cyclization of only one prenyl moiety in licoricidin (**1**). Thus, treatment of **1** with $PdCl_2$ in EtOH for 4 h at room temperature and subsequent preparative HPLC afforded the mono(2,2-dimethyl-2*H*-pyrano)isoflavans **7**–**9** in 5.1%, 3.2%, and 7.5% yields, respectively. The molecular formula of **7**, $C_{26}H_{30}O_5$, was established by HRESITOFMS, which showed an $[M - H]^-$ ion at m/z 421.2028 (calcd 421.2015), and the ^{1}H NMR, retention time, UV, and mass data were identical to those of the natural product isolated from the extract. The structure of **7** could not be determined conclusively solely on the basis of NMR data due to a lack of correlations between the phenolic protons and the B-ring carbons and to an absence of detectable NOE effects between the OH groups and aromatic protons. The structure of **7**, named licorisoflavan C, could, however, be assigned as 7,4′-dihydroxy-5-methoxy-6-prenyl-6‴,6‴-dimethylpyrano[2‴,3‴:2′,3′]isoflavan based on the feasible outcomes of the synthesis and the established identities of regioisomers **8** and **9**. Isoflavan **7** was shown to be naturally occurring based on comparison of its HPLC retention time and UV and MS data with the identical compound in the licorice extract.

The absolute configuration at C-3 for naturally occurring **7** was established by electronic circular dichroism (ECD). The positive Cotton effect (CE) near 300 nm and the negative CE

Table 2. ^{13}C NMR Spectroscopic Data for Licorisoflavans C, D, and E (7–9)

	licorisoflavan C (7)	licorisoflavan D (8)	licorisoflavan E (9)
position	δ_C, mult.[a]	δ_C, mult.[b]	δ_C, mult.[c]
2	70.3, CH$_2$	70.7, CH$_2$	69.8, CH$_2$
3	32.4, CH	31.9, CH	31.6, CH
4	26.7, CH$_2$	26.7, CH$_2$	26.5, CH$_2$
5	158.4, C	155.5, C	157.7, C
6	114.5, C	108.7, C	114.0, C
7	155.5, C	153.7, C	155.1, C
8	99.9, CH	100.9, CH	99.1, CH
9	154.5, C	156.5, C	153.8, C
10	108.3, C	109.5, C	107.7, C
1′	121.1, C	120.4, C	121.5, C
2′	152.8, C	154.1, C	150.6, C
3′	110.4, C	116.3, C	110.6, C
4′	152.2, C	155.4, C	152.8, C
5′	108.4, CH	108.3, CH	108.4, CH
6′	128.0, CH	125.2, CH	127.2, CH
1″	23.4, CH$_2$	117.8, CH	22.9, CH$_2$
2″	125.3, CH	128.5, CH	124.7, CH
3″	130.3, C	76.4, C	129.9, C
4″	25.9, CH$_3$	27.9, CH$_3$	25.5, CH$_3$
5″	17.9, CH$_3$	27.8, CH$_3$	17.5, CH$_3$
1‴	118.1, CH	23.3, CH$_2$	117.3, CH
2‴	129.3, CH	123.9, CH	129.8, CH
3‴	76.5, C	131.8, C	75.4, C
4‴	27.9, CH$_3$	25.9, CH$_3$	27.4, CH$_3$
5‴	27.9, CH$_3$	18.0, CH$_3$	27.4, CH$_3$
OCH$_3$-5	60.6, CH$_3$	61.5, CH$_3$	60.1, CH$_3$

[a]Data collected in acetone-d_6 at 125 MHz. [b]Data collected in acetone-d_6 at 100 MHz. [c]Data (150 MHz) derived from HMBC experiments in acetone-d_6.

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near 240 nm indicated the *P*-helicity of the C-ring and corresponding (3*R*) absolute configuration by analogy to data for similarly substituted isoflavan systems.[18] The negative specific rotation of **9** as compared to the positive specific rotations of **7** and **8** reflects the unreliability of specific rotation alone for assignment of absolute configuration for the isoflavans.[18] The absolute configurations for **8** and **9** could, however, be assigned as above on the basis of their ECD spectra and were established as (3*R*).[18]

Differences in the NMR spectra of compound **8** as compared to **7** and **9** reflect the effects of prenyl group cyclization onto the A-ring versus onto the B-ring, respectively. The cyclization process shifts the prenyl double bond into conjugation with the aromatic rings. Cyclization effects and such concomitant double-bond changes were most apparent in the shifts of the prenyl group protons (H-1″, H-2″, H-1‴, and H-2‴) and carbons (C-1″, C-2″, C-3″, C-1‴, C-2‴, and C-3‴) and, to a lesser extent, the aromatic protons, H-8, H-5′, and H-6′. The conjugation associated with cyclization onto the B-ring in **7** and **9** resulted in distinct shielding of C-3′ (δ 110.4 and 110.6 in **7** and **9**, respectively, versus 116.3 in **8**) and deshielding of C-6′ (δ 128.0 and 127.2 in **7** and **9**, respectively, versus 125.2 in **8**), while conjugation associated with cyclization onto the A-ring of **8** resulted in distinct shielding of C-6 (δ 108.7 in **8**, versus 114.5 and 114.0 in **7** and **9**, respectively). Cyclization involving OH-2′ in **7** versus OH-4′ in **9** was reflected in the ^{13}C NMR spectra, with slight differences in chemical shifts for C-2′ (δ 152.8 and 150.6 for **7** and **9**, respectively). Overall, **7**, **9**, kanzonol I (7-*O*-methyllicorisoflavan C), and 7-*O*-methyllicorisoflavan E had similar ^{1}H NMR characteristics,[17] providing further confirmation of the structural assignments made.

Table 3 collates the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the nine compounds tested against *P. gingivalis* and *S. mutans*. Compounds **1** and **2** were reported previously as inhibitors of the oral pathogens *P. gingivalis* and *Prevotella intermedia* at a concentration of 5 μg/mL and *S. mutans* and *S. sobrinus* at 10 μg/mL.[3] Both compounds therefore were included in the present study as additional positive controls and to assess the MIC/MBC values for these compounds. The isoflavans **7**–**9** showed marked antibacterial activities against both oral pathogens tested. MIC and MBC values of 6.25–12.5 μg/mL against *P. gingivalis* were obtained. For *S. mutans*, the MIC values of **7**–**9** were in the range 6.25–25.0 μg/mL. Compounds **7** and **9** gave MBC values of 6.25 and 12.5 μg/mL, respectively, and the MBC value for **8** was >200 μg/mL. These results suggest that isoflavans **7** and **9** may act via a bactericidal mechanism of action against both bacteria, while **8** may be bactericidal only against *P. gingivalis*.[19] Weaker activity against *P. gingivalis* was observed with isoflavan-quinone **3** and pterocarpan **4**. Compounds **3** and **4** were inactive against *S. mutans*, and **5** and **6** were inactive in both assays. Although prenylated pterocarpans reportedly have excellent antibacterial properties against *S. mutans*,[20] pterocarpan **4** showed less potent antibacterial activity than the isoflavans **1**, **2**, and **7**–**9**.

Table 3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Values for 1–9 against *P. gingivalis* and *S. mutans*

	P. gingivalis ATCC 33277		*S. mutans* ATCC 35668	
compound	MIC[a]	MBC[a]	MIC[a]	MBC[a]
1	3.125	3.125	12.5	25
2	1.56	1.56	50	100
3	25	25	>200	>200
4	50	50	200	>200
5	200	200	>200	>200
6	200	200	>200	>200
7	6.25	6.25	6.25	6.25
8	6.25	6.25	25	>200
9	12.5	12.5	12.5	12.5
tetracycline	1.56	12.5		
penicillin G			0.05	1.56

[a]MIC and MBC in μg/mL.

Previous reports suggest that some degree of lipophilicity and phenolic hydroxy groups are required for the antibacterial activity of isoflavonoids.[21] Earlier results supported this premise as isoflavans with two prenyl groups exhibited more potent antibacterial activities than isoflavans bearing only one such group.[3] In the present study, cyclization of one prenyl group onto the B-ring of compounds **7** and **9** as compared to **1** had minimal effects on activity against *S. mutans* regardless of whether ring formation involved C-2′ or C-4′. Cyclization of a single prenyl group, however, did appear to reduce the activity against *P. gingivalis* (compounds **7**–**9** as compared to **1** and **2**), irrespective of the prenyl group or aromatic ring involved.

A review on structure–activity relationships of isoflavanoids reported that the presence of a C-7 and/or C-5 free hydroxy group leads to isoflavonoids with more potent antibacterial activity.[22] As compounds **1**, **2**, and **7**–**9** all possess C-5 *O*-methyl groups, the possible contribution of the C-7 hydroxy group to antibacterial activity could be assessed. Compound **8**, with the C-7 hydroxy group involved in prenyl group cyclization, was less active than **7** or **9** against *S. mutans*, but was active against *P. gingivalis*. Compound **2**, with a C-7 *O*-methyl, also exhibited reduced activity against *S. mutans*, with potent inhibition of *P. gingivalis*. These results support the premise that a free C-7 hydroxy group is important for the activity of isoflavans against *S. mutans* and also suggest that additional factors besides lipophilicity and the presence of free hydroxy groups at C-5 and/or C-7 may contribute to the antibacterial activities of isoflavans against *P. gingivalis*.

This study demonstrates the potential benefits of licorice root extract for the treatment or prevention of major oral diseases due to the presence of isoflavans **1**, **2**, **7**, and **8**. One of the challenges with products for the oral cavity is the short time of exposure of an agent to tooth and gum surfaces. While this time period may be extended by the use of hard lozenges, candies, or teas, it remains short for products such as mouthwash or toothpaste, as exemplified by a study of 173 U.S. adults, who indicated the average time to brush the teeth was 46 s.[23] More studies are therefore needed to confirm the abilities of licorice-containing products to alter the composition of oral microflora in humans to improve oral health.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were recorded on a 589-546 Rudolph Research Analytical Autopol III automatic polarimeter. UV data were measured on a Cary 60 UV spectrophotometer (Agilent, Burlington, MA, USA). ECD data were collected using an Olis Cary-17 spectrophotometer (1 cm path length cell). NMR spectra were obtained using an AVANCE II 500 MHz or an AVANCE 600 MHz instrument (Bruker Biospin, Billerica, MA, USA). Analytical and semipreparative HPLC was carried out using an Agilent 1100 unit consisting of an Agilent quaternary pump, UV/vis detector (DAD), degasser, automatic sample injector, and Agilent

1100 series LC/MSD trap (Agilent, Burlington, MA, USA). HPLC analysis of fractions and isolates was performed on a Zorbax Eclipse XDB C_{18} column (250 × 4.6 mm i.d., 5 μm; Agilent, Santa Clara, CA, USA), with a Zorbax C_{18} guard column (12.5 × 4.6 mm i.d., 5 μm) using a flow rate of 1.0 mL/min, a column temperature of 30 °C, detection at 280 nm, and a mobile phase of MeCN (0.05% TFA)−H_2O (0.05% TFA) with 0−3 min (45:55); 3−25 min (45:55 to 100:0). All solvents for HPLC were from Fisher Scientific (Pittsburgh, PA, USA). Trifluoroacetic acid, Ac_2O, benzene, DDQ, $PdCl_2$, and pyridine were obtained from Sigma (St. Louis, MO, USA).

Plant Material. Commercially available dried and cut *G. uralensis* roots (lot # 770407, Flavex, Rehlingen, Germany) were used for this project. The identity of the material was based on comparison of the HPLC-UV trace with authentic *G. uralensis* material obtained from the American Herbal Pharmacopeia. A voucher specimen (TOM 10001) was deposited at the Tom's of Maine Herbarium in Kennebunk, ME.

Extraction and Isolation. Plant material (0.41 g) was ground and extracted using supercritical CO_2 with 5% EtOH as a modifier. The compounds described here were isolated from two fractions previously obtained by gel filtration of MPLC fraction 11 on Sephadex LH-20.[3] Sephadex fraction 3 (106 mg) was separated by semipreparative HPLC on a Zorbax SB C_{18} column (250 × 9.4 mm, 5 μm) using MeOH (0.001% TFA)−H_2O (0.001% TFA) (74:26) at 45 °C and a flow rate of 3 mL/min, yielding **1** (1.3 mg), **3** (0.9 mg), **4** (0.2 mg), **5** (0.6 mg), licoricone (0.4 mg), glyasperin D (0.6 mg), 1-methoxyficifolinol (0.2 mg), and 6,8-diprenylgenistein (1.0 mg). Sephadex fraction 4 (31 mg) was separated by semipreparative HPLC with the same column and mobile phase (ratio 73:27) at 50 °C, yielding **1** (1.9 mg), **2** (1.5 mg), **6** (0.8 mg), **7** (0.9 mg), glycyrin (1.0 mg), and glyasperin D (1.0 mg).

*Licorisoflavan C (**7**):* white powder; $[\alpha]_D^{21}$ +18 (*c* 0.1, MeOH); UV (EtOH) λ_{max} (log ε) 207 (4.71), 224 sh (4.59), 281 (4.04), 310 sh (3.40) nm; ECD (*c* 0.004, MeOH) λ ($\Delta\varepsilon$) 210 (+8.8), 238 (−5.2), 265 sh (+1.8), 289 (+4.5) nm; ^{1}H (600 MHz, acetone-d_6) and ^{13}C (150 MHz, acetone-d_6) NMR data, see Tables 1 and 2; HRESITOFMS *m/z* 423.2178 $[M + H]^+$ (calcd for $[C_{26}H_{30}O_5 + H]^+$, 423.2172); HPLC-ESIMS *m/z* 423 $[M + H]^+$, MS^2 *m/z* 367 $[M + H - 56]^+$, 221 $[M + H - 202]^+$, 189 $[M + H - 234]^+$.

*Synthesis of **8**.* Licoricidin (**1**, 40 mg) and DDQ (25 mg) were dissolved in benzene (6 mL) and heated under reflux for 5 min. After cooling, the sample was evaporated to dryness and separated by semipreparative HPLC on a Zorbax SB C_{18} column (250 × 9.4 mm, 5 μm) using MeOH−H_2O (82:18), at a flow rate of 3 mL/min at 40 °C, yielding the starting material (**1**, 2.9 mg), pterocarpan **4** (1.0 mg), and **8** (9.9 mg).

*Licorisoflavan D (**8**):* white powder; $[\alpha]_D^{21}$ +39 (*c* 0.3, MeOH); UV (EtOH) λ_{max} (log ε) 205 (4.58), 277 (3.96), 285 (2.95), 306 (3.65) nm; ECD (*c* 0.005, MeOH) λ ($\Delta\varepsilon$) 221 (+12.1), 242 (−4.3), 277 (+6.4), 286 (+7.0) nm; ^{1}H (600 MHz, acetone-d_6) and ^{13}C (150 MHz, acetone-d_6) NMR data, see Tables 1 and 2; HRESITOFMS *m/z* 423.2191 $[M + H]^+$ (calcd for $[C_{26}H_{30}O_5 + H]^+$, 423.2172); HPLC-ESIMS *m/z* 423 $[M + H]^+$, MS^2 *m/z* 367 $[M + H - 56]^+$, 231 $[M + H - 192]^+$, 219 $[M + H - 204]^+$, 191 $[M + H - 232]^+$.

*Acetylation of **1**.* Licoricidin (**1**, 254 mg) was dissolved in pyridine (25 mL), to which Ac_2O (130 mg) was added. The mixture was stirred in an airtight vial for 16 h, as preliminary studies showed that this amount of time was optimum for obtaining high yields of diacetylated licoricidin isomers. After evaporation to dryness, the residue was separated by semipreparative HPLC on an Agilent PrepHT XDB C_{18} column (250 × 21.2 mm, 7 μm) using MeOH−H_2O (88:12), at a flow rate of 6 mL/min and a temperature of 50 °C, yielding 7,2′-diacetyllicoricidin (24.2 mg), 7,4′-diacetyllicoricidin (6.7 mg), and 2′,4′-diacetyllicoricidin (54.6 mg).

7,2′-Diacetyllicoricidin: white powder; ^{1}H NMR (600 MHz, acetone-d_6) δ 8.57 (1H, br s, OH-4′), 7.05 (1H, d, *J* = 8.4 Hz, H-6′), 6.83 (1H, d, *J* = 8.4 Hz, H-5′), 6.37 (1H, s, H-8), 5.17 (1H, m, H-2‴), 5.08 (1H, m, H-2″), 4.13 (1H, ddd, *J* = 2.0, 3.0, 10 Hz, H-2α), 3.94 (1H, m, H-2β), 3.70 (3H, s, OCH_3-5), 3.23 (2H, dd, *J* = 6.9, 15 Hz, H-1″b, H-1‴b), 3.17 (2H, dd, *J* = 6.5, 15 Hz, H-1″a, H-1‴a), 3.07 (1H, m, H-3), 2.96 (1H, ddd, *J* = 1.9, 5.0, 16 Hz, H-4α), 2.80 (1H, dd, *J* = 11, 16 Hz, H-4β), 2.32 (3H, s, CH_3COO-2′), 2.23 (3H, s, CH_3COO-7), 1.76 (3H, s, H_3-5‴), 1.74 (3H, s, H_3-5″), 1.65 (6H, s, H_3-4″, H_3-4‴); ^{13}C NMR (150 MHz, acetone-d_6) 169.9 (C, CH_3COO-2′), 169.5 (C, CH_3COO-7), 158.0 (C, C-5), 155.6 (C, C-4′), 154.1 (C, C-9), 149.4 (C, C-7), 149.1 (C, C-2′), 131.6 (CH, C-2‴), 131.3 (CH, C-2″), 125.8 (CH, C-6′), 125.7 (C, C-1′), 124.0 (C, C-3″), 123.1 (C, C-3‴), 121.9 (C, C-3′), 119.7 (C, C-6), 115.0 (C, C-10), 114.0 (CH, C-5′), 107.6 (CH, C-8), 70.4 (CH_2, C-2), 60.7 (CH_3, OCH_3-5), 31.9 (CH, C-3), 27.8 (CH_2, C-4), 25.8 (CH_3, C-4‴), 25.7 (CH_3, C-4″), 24.2 (CH_2, C-1‴), 24.0 (CH_2, C-1″), 20.6 (CH_3, CH_3COO-7, CH_3COO-2′) 17.9 (CH_3, C-5″, C-5‴).

7,4′-Diacetyllicoricidin and 2′,4′-Diacetyllicoricidin. See Supporting Information.

*Synthesis of Licorisoflavan E (**9**).* 7,2′-Diacetyllicoricidin (16.2 mg) was dissolved in 90% aqueous EtOH (1.0 mL), and $PdCl_2$ (5.9 mg) was added. The mixture was stirred at room temperature for 48 h, the solvent was evaporated, and the residue was dissolved in 1.2 N NaOH (1 mL) in 90% aqueous EtOH for 30 min to hydrolyze the acetate groups. Licorisoflavan E (**9**; 1.5 mg) was obtained after purification by semipreparative HPLC on an Agilent PrepHT XDB C_{18} column (250 × 21.2 mm, 7 μm) using MeOH−H_2O (91:9), at a flow rate of 6 mL/min and a temperature of 50 °C.

*Licorisoflavan E (**9**):* white powder; $[\alpha]_D^{21}$ −9 (*c* 0.1, MeOH); UV (EtOH) λ_{max} (log ε) 207 (4.73), 227 (4.64), 279 (4.09), 309 sh (3.43) nm; ECD (*c* 0.004, MeOH) λ ($\Delta\varepsilon$) 218 (+10.0), 239 (−3.9), 262 (+1.7), 273 (+1.7), 286 (+1.8) nm; ^{1}H (600 MHz, acetone-d_6) and ^{13}C (150 MHz, acetone-d_6) NMR data, see Tables 1 and 2; HPLC-ESIMS *m/z* 423 $[M + H]^+$, MS^2 *m/z* 367 $[M + H - 56]^+$, 221 $[M + H - 202]^+$, 189 $[M + H - 234]^+$.

*Synthesis of Licorisoflavan C (**7**).* Licoricidin (**1**, 102 mg) was dissolved in EtOH (2.0 mL), to which $PdCl_2$ (18.4 mg) was added. The mixture was stirred at room temperature for 4 h and directly subjected to semipreparative fractionation by HPLC on an Agilent PrepHT XDB C_{18} column (250 × 21.2 mm, 7 μm) using MeCN−H_2O (88:12), at a flow rate of 5.6 mL/min and a temperature of 40 °C, to yield **7** (5.2 mg), **8** (3.2 mg), and **9** (7.6 mg).

Bioassays. Stock solutions of compounds were prepared in EtOH and kept at 4 °C protected from light. Minimal inhibitory concentrations and minimal bactericidal concentrations were determined using a microbroth dilution assay. Briefly, *S. mutans* (ATCC 35668) and *P. gingivalis* (ATCC 33277) were grown at 37 °C in Todd-Hewitt broth (THB) (BBL Microbiology Systems, Cokeysville, MD, USA) supplemented with hemin (10 μg/mL) and vitamin K (10 μg/mL). *P. gingivalis* and *S. mutans* were incubated under anaerobic (N_2/H_2/CO_2: 80/10/10) and aerobic conditions, respectively. Overnight cultures were diluted in fresh THB medium to obtain an optical density at 660 nm (OD_{660}) of 0.2. Equal volumes (100 μL) of bacteria and a series of 2-fold dilutions of compounds (200 μg/mL to 0.78 μg/mL) in culture medium were mixed into the wells of 96-well plates (Sarstedt, Newton, NC, USA). Control wells with no bacteria or no compounds were also prepared. Penicillin G and tetracycline were used as reference compounds for *S. mutans* and *P. gingivalis*, respectively. After an incubation of 24 h at 37 °C, bacterial growth was recorded visually. MIC values (μg/mL) of compounds for each bacterial species were determined as the lowest concentration at which no growth occurred. To determine MBC values (μg/mL), aliquots (5 μL) of each well showing no visible growth were spread on THB agar plates, which were incubated for 3 days at 37 °C. MBCs of compounds for each bacterial species were determined as the lowest concentration at which no colony formation occurred. The MIC and MBC values were determined in three independent experiments to assess reproducibility.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR spectra of 7,2′-diacetyllicoricidin, and **7**−**9**, as well as NMR data and spectra of 7,4′-diacetyllicoricidin and 2′,4′-diacetyllicoricidinare available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel: +1 207-985-2944. Fax: +1 207-985-2196. E-mail: sgafner99@gmail.com.

Notes

The authors declare no competing financial interest.

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DEDICATION

Dedicated to Prof. Dr. Otto Sticher, of ETH-Zurich, Zurich, Switzerland, for his pioneering work in pharmacognosy and phytochemistry.

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