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Isoflavonoids and Coumarins from *Glycyrrhiza uralensis*: Antibacterial Activity against Oral Pathogens and Conversion of Isoflavans into Isoflavan-Quinones during Purification

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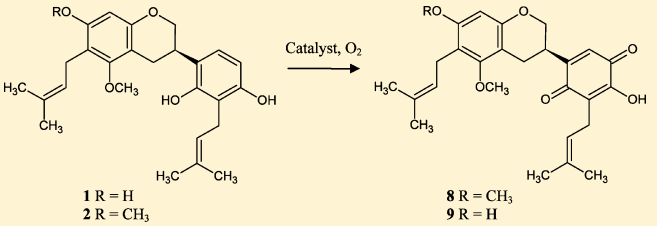
Supporting Information

ABSTRACT: Phytochemical investigation of a supercritical fluid extract of *Glycyrrhiza uralensis* has led to the isolation of 20 known isoflavonoids and coumarins, and glycycarpan (**7**), a new pterocarpan. The presence of two isoflavan-quinones, licoriquinone A (**8**) and licoriquinone B (**9**), in a fraction subjected to gel filtration on Sephadex LH-20 is due to suspected metal-catalyzed oxidative degradation of licoricidin (**1**) and licorisoflavan A (**2**). The major compounds in the extract, as well as **8**, were evaluated for their ability to inhibit the growth of several major oral pathogens. Compounds **1** and **2** showed the most potent antibacterial activities, causing a marked growth inhibition of the cariogenic species *Streptococcus mutans* and *Streptococcus sobrinus* at 10 μg/mL and the periodontopathogenic species *Porphyromonas gingivalis* (at 5 μg/mL) and *Prevotella intermedia* (at 5 μg/mL for **1** and 2.5 μg/mL for **2**). Only **1** moderately inhibited growth of *Fusobacterium nucleatum* at the highest concentration tested (10 μg/mL).



Chinese licorice (*Glycyrrhiza uralensis* Fisch., Fabaceae) is found in steppe meadows, at lake and river sides, in ravines, on slopes, and in depressions throughout much of northern mainland China, especially the Asian steppes to the west. While *G. uralensis* is the main species used in Asia, European licorice (*Glycyrrhiza glabra* L.) also occurs in northwest China, in wild desert regions, dry plains, grassy plains with salty alkaline soil, and fallow wastelands that were once used for producing rice, wheat, and millet. These two species along with another Chinese native, *Glycyrrhiza inflata* Bat., are official drug plants in the *Chinese Pharmacopoeia*. The Chinese call licorice "gan-cao", which means "sweet herb". Licorice is used in many Chinese herbal prescriptions as a guide drug to enhance the activity of other ingredients, reduce toxicity, and improve flavor.[1] The major constituents of licorice are the triterpene glycosides, which give the roots its typical sweet taste. The aglycones are oleanane-type pentacyclic triterpenes with glycyrrhetinic acid as the best known example.[2] Another important class of compounds is the flavonoids, of which more than 300 have been characterized so far in licorice species. The major flavonoids belong to the flavanone and chalcone type, but the lipophilic fraction also contains rather unusual flavonoids such as isoflavans, isoflavenes, and pterocarpans.[1,2] Less common phenolic compounds include coumarins, 2-arylbenzofurans, and coumestans. Polysaccharides have been found in *G. uralensis* and *G. glabra* and reportedly constitute up to 10% of the roots of *G. glabra*.[2,3]

Despite the extensive use of licorice in traditional medicine, there has been limited evidence for its use to treat ailments of the oral cavity. Research by Martin and co-workers[4] has focused on the benefits of a water extract of licorice root for the treatment of aphthous ulcers. There are also two reports on the ability of glycyrrhizin, the sweet component of licorice root, to reduce dental plaque formation;[5,6] however, this effect could not be confirmed in a small clinical trial.[7] In addition, He and co-workers[8] reported that pterocarpenes isolated from *G. uralensis* roots exerted antibacterial activity against *Streptococcus mutans*, the major etiologic agent of dental caries. Our previous studies have focused on a supercritical fluid extract of *G. uralensis* roots, which has shown potent anti-inflammatory properties in an in vitro macrophage model and an ex vivo human whole blood model.[9,10] More specifically, the extract

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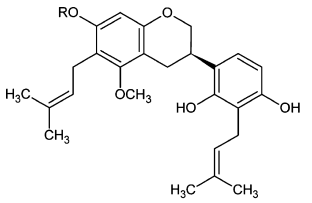
Chart 1



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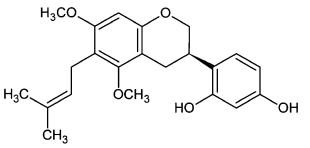
2 R = CH_3



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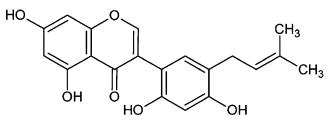

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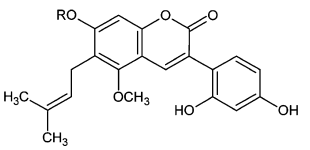
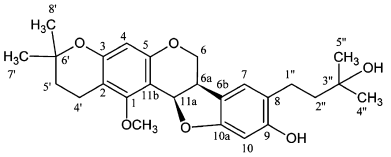
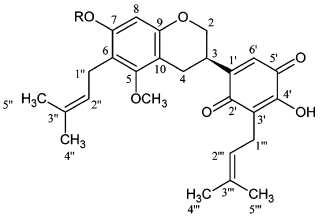


5 R = CH_3
6 R = H



7



8 R = CH_3
9 R = H

inhibited lipopolysaccharide (LPS)-induced secretion of pro-inflammatory cytokines, chemokines, and matrix metalloproteinases by macrophages. This effect was at least in part due to the reduced phosphorylation of key intracellular kinases. Licoricidin (**1**) and licorisoflavan A (**2**), the two predominant compounds in the extract, were isolated previously and related to at least part of the anti-inflammatory effect.[10] Herein, the identification of the minor compounds of the extract and of two isoflavan-quinones formed from licoricidin (**1**) and licorisoflavan A (**2**) during the isolation process is described. In addition, the antibacterial activities of the licorice isolates toward important cariogenic and periodontopathogenic bacterial species were compared.

RESULTS AND DISCUSSION

Fractionation of the supercritical fluid extract of *G. uralensis* roots led to the characterization of 20 known compounds and one new pterocarpan. The known compounds were identified as licoricidin (**1**),[11] licorisoflavan A (**2**),[12] vestitol,[13] glyasperin C,[14] glyasperin D[14] (**3**), glyasperin F,[15] dihydrolicoisoflavone,[15] allolicoisoflavone A (**4**),[16] semilicoisoflavone B,[17] licoricone,[18] medicarpin,[19] edudiol,[20] 1-methoxyficifolinol,[17] kanzonol P,[21] glycyrin (**5**),[14] licocoumarone,[14,18] gancaonin I,[18] glycycoumarin (**6**),[22] and licoarylcoumarin,[23] based on comparison of spectroscopic and spectrometric information with published data. Liquiritigenin was identified on the basis of comparison of HPLC retention time, UV, and MS data with a commercially available standard compound.

The molecular formula of a new pterocarpan (**7**), $C_{26}H_{32}O_6$, was determined by HREIMS, which showed a molecular ion peak at *m*/*z* 440.2205 (calcd 440.2199). The pterocarpan skeleton was deduced from the NMR data. In the ^{1}H NMR spectrum, the signals at δ 4.14 (dd, J = 11.5, 5.0 Hz), 3.60 (t, J = 11.1 Hz), 3.35 (m), and 5.61 (d, J = 6.7 Hz), corresponding to H-6*α*, H-6*β*, H-6a, and H-11a, respectively, were similar to the signals of the same protons present in the ^{1}H NMR spectra of known pterocarpans isolated from the extract. In addition, the ^{1}H NMR spectrum contained signals for three aromatic protons (δ 6.93, 6.41, and 6.21, all s), one methoxy group (δ 3.95, s), and two oxygenated 3-methylbutyl units [δ 2.67 (m), 1.79 (m), 1.30 (s), 1.26 (s) and 2.79 (m), 1.79 (m), 1.36 (s), 1.32 (s), respectively].

The location of the substituents on the two aromatic rings of **7** was based mainly on the ^{13}C NMR data and HMBC correlations. HMBC correlations were observed between H-11a and C-1 (δ 159.3) as well as the methoxy protons and C-1, placing the methoxy group at C-1. On the basis of the ^{13}C NMR shift of the methoxy carbon, indicating a di-*ortho* substitution, and the HMBC correlation between H-4′ (δ 2.79, m) and C-1 and C-3 (δ 156.3), one of the isoprene groups had to be attached at C-2 (δ 108.4), with C-3 found to bear an oxygenated substituent. The incorporation of the 3-methylbutyl side chain into a 6,6-dimethyl-4,5-dihydro-[6*H*]-pyran ring was established on the basis of the ^{13}C NMR shifts. One aromatic proton was placed at C-4, while the two remaining aromatic protons were located at C-7 and C-10 due to the lack of a discernible coupling. The HMBC correlations between H-7 and C-6a (δ 39.1), C-1″ (δ 24.2), C-9 (δ 154.9), and C-10a (δ 159.1) proved that the second 3-methylbutyl unit was attached at C-8 (δ 120.6), with an oxygenated aromatic carbon adjacent at C-9. The ^{13}C NMR shifts of the side chain were consistent with a 3-hydroxy-3-methylbutyl moiety. Thus, the structure of **7** was established as 9-hydroxy-1-methoxy-8-(3-hydroxy-3-methylbutyl)-6′,6′-dimethyl-4′,5′-dihydro-[6*H*]-pyrano[2′,3′:3,2]-

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Table 1. NMR Spectroscopic Data of Licoriquinone A (8) and Licoriquinone B (9)

	licoriquinone A (8)			licoriquinone B (9)		
position	δ_C, mult.	δ_H (J in Hz)	HMBC[a]	δ_C, mult.	δ_H (J in Hz)	HMBC[a]
2	68.2, CH_2	3.92, dd (10.6, 7.9) 4.20, ddd (10.6, 3.1, 1.6)	3,4,9,1′	68.1, CH_2	3.88, dd (10.5, 7.7) 4.16, ddd (10.5, 3.1, 1.4)	3,4,9,1′
3	31.0, CH	3.33, ddddd (8.6, 7.9, 5.6, 3.1, 1.1)	2,4,6′	30.9, CH	3.31, ddddd (8.6, 7.7, 5.5, 3.1, 1.1)	2,4,10,1′
4	24.5, CH_2	2.65, dd (16.2, 8.6) 2.90, ddd (16.2, 5.6, 1.6)	2,3,5,6,9,10,1′	24.3, CH_2	2.63, dd (16.2, 8.6) 2.88, ddd (16.2, 5.5, 1.4)	2,3,5,9,10,1′
10	106.0, C			106.4, C		
5	156.9, C			157.3, C		
6	116.3, C			114.1, C		
7	157.6, C			154.2, C		
8	95.9, CH	6.23, s	4,6,7,9,10,1″	99.1, CH	6.09, s	4,5,6,7,9,10,1″
9	153.2, C			153.0, C		
1′	150.5, C			150.3, C		
2′	186.4, C			186.4, C		
3′	120.9, C			120.5, C		
4′	151.5, C			151.4, C		
5′	183.4, C			183.5, C		
6′	128.0, CH	6.44, d (1.1)	2,3,1′,2′,4′	128.4, CH	6.44, d (1.1)	2,3,1′,2′,4′
1″	22.7, CH_2	3.21, d (7.3)	5,6,7,2″,3″	22.2, CH_2	3.20, d (6.8)	5,6,7,2″,3″
2″	123.7, CH	5.11, ddt (7.3, 2.9, 1.2)	4″,5″	123.6, CH	5.13, dt (6.8, 1.2)	4″,5″
3″	130.9,			130.7, C		
4″	C25.8, CH_3	1.63, d (1.2)	2″,3″, 5″	25.2, CH_3	1.65, m	2″,3″, 5″
5″	17.8, CH_3	1.73, s	2″,3″,4″	16.8, CH_3	1.72, s	2″,3″,4″
1‴	22.3, CH_2	3.10, d (7.1)	4′,2′,2‴,3‴	21.7, CH_2	3.09, d (7.1)	4′,2′,2‴,3‴
2‴	119.6, CH	5.07, dt (7.1, 1.2)	4‴,5‴	120.3, CH	5.08, dt (7.1, 1.1)	4‴,5‴
3‴	133.9, C			132.8, C		
4‴	25.8, CH_3	1.65, d (1.2)	2‴,3‴,5‴	25.2, CH_3	1.65, m	2‴,3‴,5‴
5‴	17.8, CH_3	1.71, s	2‴,3‴,4‴	16.8, CH_3	1.72, s	2‴,3‴,4‴
OCH_3-5	60.7, CH_3	3.70, s	5	60.0, CH_3	3.64, s	5
OCH_3-7	55.6, CH_3	3.77, s	7			

[a]HMBC correlations, optimized for 8 Hz, are from proton(s) to the indicated carbon.

pterocarpan. The compound has been named glycycarpan. The absolute configuration of the compound was determined from its optical rotation. Levorotary pterocarpans have the 6a*R*, 11a*R* absolute configuration, and dextrorotary ones are 6a*S*, 11a*S*.[24] The $[\alpha]_D^{21}$ of −35.3 measured for **7** is consistent with a 6a*R*, 11a*R* configuration.

In an attempt to isolate a larger amount of **2**, a fraction rich in this compound was subjected to gel filtration on Sephadex LH-20. Besides a number of mixed fractions, there was one fraction containing **2** and a small amount of compound **8**, both of which were purified by semipreparative HPLC. The UV spectrum of **8**, with maxima at 274 and 386 nm, suggested that the compound is highly oxygenated. The molecular formula was established as $C_{27}H_{32}O_6$, on the basis of the HRTOFMS data with an $[M + H]^+$ peak at m/z 453.2241 (calcd 453.2272). The ^{1}H NMR signals at δ 4.20 (ddd, J = 10.6, 3.1, 1.6 Hz, H-2α), 3.92 (dd, J = 10.6, 7.9 Hz, H-2β), 3.33 (ddddd, J = 8.6, 7.9, 5.6, 3.1, 1.1 Hz, H-3), 2.90 (ddd, J = 16.2, 5.6, 1.6 Hz, H-4α), and 2.65 (dd, J = 16.2, 8.6 Hz, H-4β) are typical for the C ring of an isoflavan. In addition, the spectrum revealed signals for two aromatic protons, two methoxy groups, and two isoprenyl moieties (Table 1). The HMBC data indicated that the substitution pattern on the A ring was the same as in **2**. The correlation between H-6′ (δ 6.44) and C-3 (δ 31.0) and C-2′ (δ 186.4), the UV spectrum, and consideration of the molecular formula suggested the presence of an isoflavan-quinone ring, but the lack of proper ^{13}C NMR data initially prevented the unequivocal elucidation of the structure.

As part of our conducted stability studies, compounds **1** and **2** were subjected to stress tests under alkaline, acidic, and oxidative conditions. Both compounds were unstable in 0.1 N NaOH, yielding two major degradation products. One of the degradation products derived from **2** had the same retention time, UV, and MS data as **8**. In order to confirm the structure of **8**, a larger amount of **2** was treated with 0.1 N NaOH for 2 h, and the resulting mixture was analyzed by HPLC-NMR. The ^{13}C NMR spectrum revealed the presence of two carbonyl carbons at δ 186.4 and 183.4. The shifts of the two carbonyl carbons are in good agreement with a *para*-quinone moiety. The substitution pattern of the *para*-quinone ring was mainly based on HMBC data (Table 1), in particular the correlations between H-6′ and C-2 (δ 68.2), C-3 (δ 31.0), C-2′ (δ 186.4), and C-4′ (δ 151.5) and between H-1‴ (δ 3.10) and C-2′ and C-4′. Thus, compound **8** was identified as 4′-hydroxy-5,7-dimethoxy-6,3′-diprenylisoflavanquinone (licoriquinone A).

Compound **1** was treated in a similar manner with 0.1 N NaOH, and the resulting mixture was also subjected to HPLC-NMR. The major degradation product from **1** was identified as 4′,7-dihydroxy-5-methoxy-6,3′-diprenylisoflavanquinone (**9**) and named licoriquinone B. The configuration at C-3 of **8** and **9** was established as 3*R*, based on the specific rotation of **1** ($[\alpha]_D^{23}$ +12.8, *c* 0.3 in MeOH) and **2** ($[\alpha]_D^{23}$ +11.9, *c* 0.4 in MeOH). Fukai et al. established the configuration of licoricidin ($[\alpha]_D^{21}$ +22.8) as 3*R* using CD data.[11] As both **8** and **9** are oxidative degradation products of **2** and **1**, and the order of the substituents at the stereogenic center does not change in the

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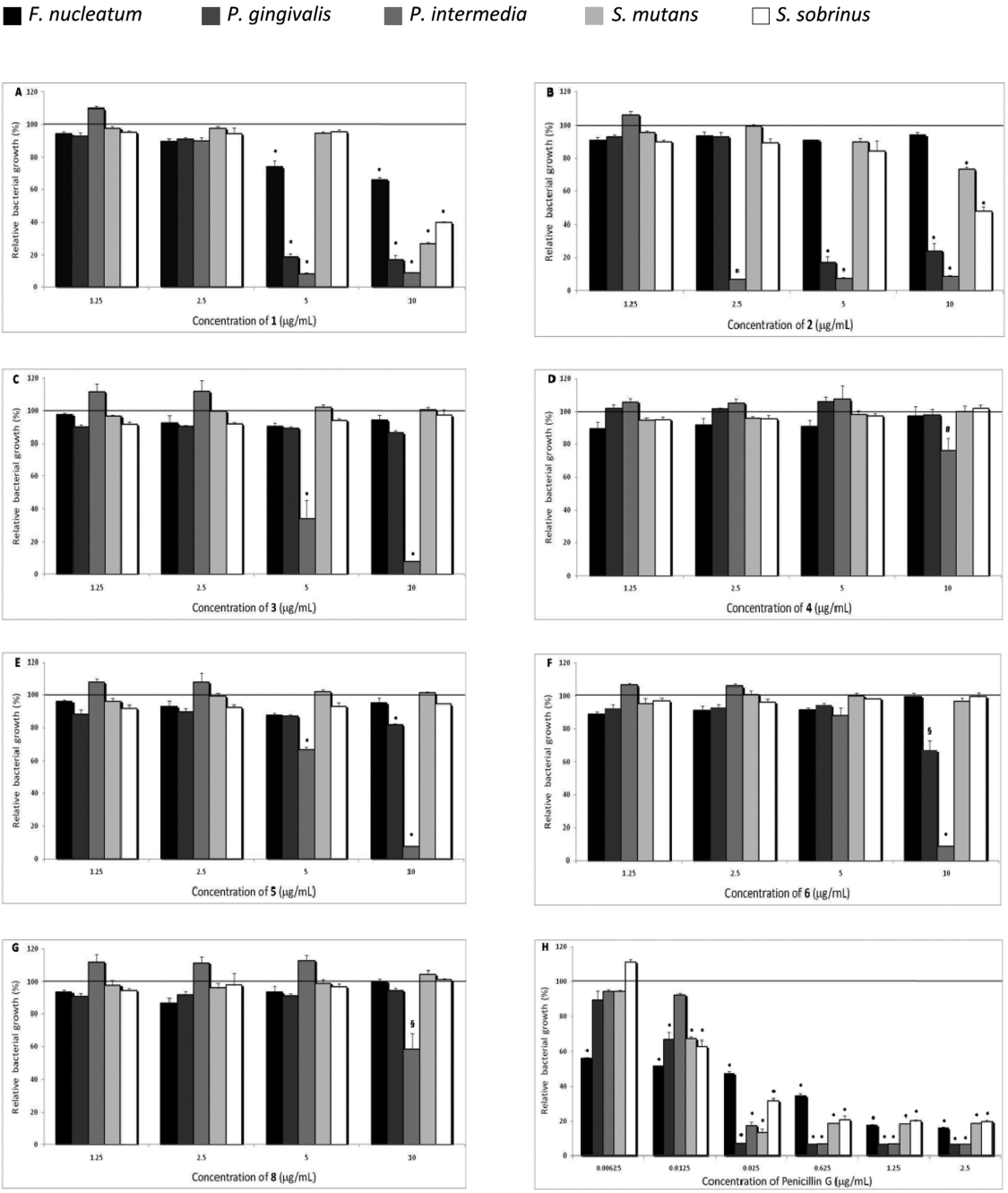

Figure 1. Effect of licorice compounds **1** (panel A), **2** (panel B), **3** (panel C), **4** (panel D), **5** (panel E), **6** (panel F), **8** (panel G), and penicillin G (panel H) on growth of *F. nucleatum*, *P. gingivalis*, *P. intermedia*, *S. mutans*, and *S. sobrinus*. The data are means ± standard deviations of triplicate assays. *, $p < 0.001$; §, $p < 0.005$; and #, $p < 0.01$ compared to control with no compounds.

oxidation process, they all have the same 3*R* configuration. Despite considerable efforts, we were unable to get unambiguous CD spectra for **1**, **2**, or **8**.

The isoflavan-quinones represent a rare group of plant secondary metabolites, and only a few structures have been reported thus far. Examples include claussequinone, astragaluquinone, pendulone, amorphaquinone, colutequinone, laurentiquinone, mucroquinone, and the abruquinones.[25−32] It is not clear as to whether isoflavan-quinones are genuine natural products or oxidation products of related isoflavans. Grosvenor and Gray[29] showed that colutequinone was present in the original extract, together with colutehydroquinone, which is readily converted into the quinone. The fact that neither **8** nor **9** was found in the supercritical fluid extract, and appeared only after a gel filtration step, indicates that certain isoflavans are prone to oxidative degradation into isoflavan-quinones. Usually, only 1,2- and 1,4-dihydroxy-substituted aromatic rings are subject to oxidation into *ortho*- and *para*-quinones, but Philipp and Schink[33] reported that resorcinol (1,3-dihydroxybenzene) oxidized into 2-hydroxy-1,4-benzoquinone under alkaline conditions with an electron acceptor having a high redox potential, e.g., $K_3Fe(CN)_6$. Furthermore, Ling et al.[34] reported that *tert*-butylresorcinols tend to autoxidize in the presence of copper(II) and that this reaction is favored by alkaline conditions. Accordingly, the proposed mechanism of oxidation of isoflavans **1** and **2** into the quinones **9** and **8** is outlined in

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Figure S1, Supporting Information. It is still not clear whether the same mechanisms led to the formation of the isoflavan-quinones during the isolation process. The fraction submitted to gel filtration did not contain detectable amounts of isoflavan-quinones; therefore, the conversion must have happened during the separation or the subsequent drying process. Due to the fact that the Sephadex LH-20 material was extensively used prior to this separation, it is possible that metal ions such as copper(II) were present on the column. Therefore, we hypothesize that the reaction may occur under neutral conditions or that an alkaline contaminant from previous use was present on the stationary phase. In order to rule out an oxidation due to the stationary phase itself or to the solvents, pure **1** or pure **2** was again submitted to gel filtration using a new lot of Sephadex LH-20. With the new material, none of the quinones were found, neither when fractions were verified directly after eluting off the column nor after one week in the CH_2Cl_2–MeOH mixture. This supports the hypothesis that the degradation was due to a contaminant from prior work with the old lot of Sephadex LH-20 used in the initial purification step. Interestingly, all the natural isoflavan-quinones possess either a hydroxy or a methoxy group at C-3′ and/or C-4′ and, therefore, may be produced from naturally occurring 2′,4′- or 3′,5′-dihydroxyisoflavans either in the plant or during isolation.

In continuation of our studies aimed to demonstrate the beneficial properties of licorice for oral health, the effects of compounds **1**–**6** and **8** (other isolates were not available in sufficient quantities to perform the testing) were examined on the growth of oral pathogens at concentrations between 1.25 and 10 μg/mL. Dental caries is the direct result of enamel dissolution by acid-producing bacteria inhabiting dental biofilm, especially when the biofilm reaches a critical mass due to poor oral hygiene. *Streptococcus mutans* and *S. sobrinus* are known as major cariogenic bacteria.[35] While compounds **3**–**6** and **8** had no effect on the growth of *S. mutans* and *S. sobrinus*, compounds **1** and **2** at 10 μg/mL caused a marked growth inhibition of both cariogenic bacterial species (Figure 1). Periodontitis is a destructive inflammatory disorder that leads to the loss of tooth support. It is initiated by a specific group of Gram-negative anaerobic bacteria, including *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Fusobacterium nucleatum*, which modulate periodontal tissue destruction through complex interactions with mucosal and immune cells.[36,37] Growth inhibition of *F. nucleatum* was observed only with compound **1** (Figure 1A). On the other hand, the growth of *P. intermedia* was inhibited by compounds **1**–**3**, **5**, and **6**, and to a lesser extent by **4** and **8** (Figure 1). Finally, complete growth inhibition of *P. gingivalis* was obtained with **1** and **2** (Figures 1A and B). Penicillin G was used as positive control and was found to be active on all bacterial species tested (Figure 1G). In conclusion, licorice compounds **1** and **2** were the most effective in inhibiting growth of oral pathogens, thus suggesting that they may have a therapeutic/preventive potential for oral infections.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-1010 polarimeter (compound **7**) or on a 589–546 Rudolph Research Analytical Autopol IV automatic polarimeter (compounds **1** and **2**). UV data were measured on a Cary 50 UV-spectrophotometer (Varian, Palo Alto, CA, USA). NMR spectra were obtained on an AVANCE II 500 MHz or an AVANCE 600 MHz instrument (Bruker Biospin, Billerica, MA, USA). The HREIMS data for **7** were obtained with an MSRoute instrument (JEOL, Peabody, MA, USA). HRMS data for **8** and **9** were obtained using the HPLC-SPE-NMR/TOFMS system described below. MPLC was conducted on a Büchi C-615 system, equipped with two C-605 pumps, a C-630 monitor, and a C-660 fraction collector (Büchi, Flawil, Switzerland). Semipreparative HPLC was carried out using an Agilent 1100 unit equipped with a DAD detector (Agilent, Burlington, MA, USA). The HPLC-SPE-NMR/TOFMS system used for this study consisted of an Agilent 1200 HPLC system (quaternary pump, autosampler, diode array detector, Waldbronn, Germany), a Bruker/Spark Prospekt 2 SPE cartridge exchanger (Emmen, The Netherlands), a MicrOTOF mass spectrometer (Bruker Daltonics, Bremen, Germany), and a 600 MHz AVANCE II NMR spectrometer equipped with a 5 mm TCI cryoprobe (Bruker Biospin, Rheinstetten, Germany). Chromatography was performed on an XSelect CSH C_{18} column (250 × 4.6 mm, 5 μm). The solvent composition started with 50% A (H_2O–0.1% formic acid-d_2) and 50% B (MeCN). On holding for 5 min, the composition changed to 100% B at 25 min using a linear gradient. The reaction mixture (30 μL) reconstituted in 1 mL of EtOH was injected onto the column. The mass spectrometer was operated in the positive ionization mode using a scan range between m/z 50 and 1000. The calibration was done with a 20 mM lithium formate solution that was introduced to the ion source with the help of a divert valve at the beginning of each chromatographic run. SPE cartridges (10 × 2 mm) filled with Hysphere C_{18} HD material were used to trap the chromatographic fractions. For the measurements of NMR data, standard parameter sets created for the Bruker SELU program (SELU Topspin 3.1) were employed uniformly (see Supporting Information). Gradient COSY and HMBC were acquired using 4 k complex data points in F2 and 512 points in the F1 dimension. The multiplicity-edited gradient HSQC was acquired with 2 k data points in F2 and 400 points in the F1 dimension. The number of scans per increment differed according to sample and experimental requirements. For **9**, the COSY was acquired with four scans, the HSQC with eight scans, and the HMBC with 32 scans per increment. For **8**, the concentration was lower, which resulted in longer acquisition times (COSY: 16 scans; HSQC: 32 scans; HMBC: 128 scans). For **9**, a ^{13}C NMR spectrum with composite pulse decoupling on the proton channel was acquired on an AVANCE III 600 MHz NMR spectrometer equipped with a 5 mm QNP cryoprobe head (Bruker Biospin, Rheinstetten, Germany). A total of 24 576 scans were collected in 131 072 complex data points at a sweep width of 36 057 Hz with a relaxation delay of 5 s. The experiment time was 26.5 h.

Reagents. LiChrosolv LCMS-grade MeCN and H_2O were obtained from Merck (Darmstadt, Germany). All other solvents for HPLC, MPLC, or forced degradation studies were from Fisher Scientific (Pittsburgh, PA, USA). TFA was purchased from Sigma (St. Louis, MO, USA). Formic acid was from Fluka (Buchs, Switzerland). Formic acid-d_2 (95%) and methanol-d_4 (99.8%) were obtained from Deutero GmbH (Kastellaun, Germany).

Plant Material. Commercially available dried and cut *Glycyrrhiza 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, USA.

Extraction and Isolation. The plant material was ground and extracted using supercritical CO_2 with 5% EtOH as a modifier. For the isolation process, see Supporting Information.

*Glycycarpan (**7**):* white powder; $[\alpha]_D^{21}$ −35.3 (*c* 1.1, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 209 (4.49), 275 (3.61) nm; ^1H NMR ($CDCl_3$, 600 MHz) δ 6.93 (1H, s, H-7), 6.41 (1H, s, H-10), 6.21 (1H, s, H-4), 5.61 (d, J = 6.7 Hz, H-11a), 4.14 (dd, J = 11.5, 5.0 Hz, H-6α), 3.95 (3H, s, OCH_3), 3.60 (t, J = 11.1 Hz, H-6β), 3.35 (1H, m, H-6a), 2.79 (2H, m, H-4′), 2.67 (2H, m, H-1″), 1.79 (2H, m, H-5′), 1.79 (2H, m, H-2″), 1.36[a] (3H, s, H-7′), 1.32[a] (3H, s, H-8′), 1.30[b] (3H, s, H-4″), 1.26[b] (3H, s, H-5″); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.3 (C, C-1), 159.1 (C, C-10a), 156.3 (C, C-3), 155.1 (C, C-5), 154.9 (C, C-9), 125.0 (CH, C-7), 120.6 (C, C-8), 118.7 (C, C-6b), 108.4 (C, C-2), 106.6 (C, C-11b), 100.8 (CH, C-4), 98.8 (CH, C-10), 75.5 (CH, C-11a), 74.6 (C, C-6′), 71.2 (C, C-3″), 66.3 (CH_2, C-6), 61.4 (CH_3,

OCH_3), 43.3 (CH_2, C-2″), 39.1 (CH, C-6a), 32.3 (CH_2, C-5′), 29.5 (CH_3, C-4″), 29.5 (CH_3, C-5″), 26.9 (CH_3, C-7′ or C-8′), 26.2 (CH_3, C-7′ or C-8′), 24.2 (CH_2, C-1″), 16.8 (CH_2, C-4′). [a,b]Signals with the same superscript are interchangeable

*Licoriquinone A (**8**):* orange powder; UV (EtOH) λ_{max} (log ε) 209 (4.52), 274 (3.72), 386 (2.83) nm; ^1H and ^{13}C NMR data, see Table 1.

*Licoriquinone B (**9**):* ^1H and ^{13}C NMR data, see Table 1.

Forced Degradation Study of 2 in 0.1 N NaOH. Compound **2** (15.9 mg) was dissolved in 9.0 mL of EtOH, to which 1.0 mL of 1 N NaOH was added. After 2 h, the reaction was quenched by adding 1.0 mL of 1 N HCl, and the solution was evaporated to dryness. The dry mixture containing **8** as the predominant degradation product was washed three times with 10 mL of deionized H_2O to remove NaCl, then freeze-dried, and finally analyzed by HPLC-NMR/MS.

Compound **1** (45.1 mg) was subjected to the same treatment in 0.1 N NaOH to give a mixture of degradation products with **9** as the predominant compound. This mixture was also subjected to HPLC-NMR/MS analysis as outlined above.

Effects on Bacterial Growth. The effects of licorice compounds **1**–**6** and **8** on the growth of Gram-negative (*P. gingivalis* ATCC 33277, *P. intermedia* ATCC 25611, *F. nucleatum* ATCC 25586) and Gram-positive (*S. mutans* ATCC 25175, *S. sobrinus* ATCC 33478) oral pathogens were determined in a microplate dilution assay. Twenty-four-hour cultures of bacteria in Todd Hewitt Broth (BBL Microbiology Systems, Cockeysville, MD, USA), supplemented with hemin (10 μg/mL) and vitamin K (1 μg/mL), were diluted in fresh broth medium to obtain an optical density at 660 nm (OD_{660}) of 0.2. Samples (100 μL) were added to the wells of a 96-well tissue culture plate (Sarstedt, Newton, NC, USA) containing 100 μL of serial dilutions of sterile compounds (10 to 1.25 μg/mL) or penicillin G (2.5 to 0.00625 μg/mL) in broth medium. Control wells with no compounds were also inoculated. After incubation for 24 h at 37 °C under anaerobic (N_2–H_2–CO_2 75:10:15) (*P. gingivalis*, *P. intermedia*, *F. nucleatum*) or aerobic (*S. mutans*, *S. sobrinus*) conditions, the OD_{660} was recorded. The means ± standard deviations of triplicate assays were calculated. When the growth inhibition was ≥20%, differences between the control and test values were analyzed for statistical significance using the Student's *t* test and were considered significant at $p < 0.01$, 0.005, or 0.001.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

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