Binaphthalenone Glycosides from African Chewing Sticks, *Diospyros lycioides*

Xing-Cong Li,[†] Pieter van der Bijl,[‡] and Christine D. Wu*[,§]

Department of Periodontics, The University of Illinois at Chicago, College of Dentistry, Chicago, Illinois 60612-7212, and Oral and Dental Research Institute, Faculty of Dentistry, University of Stellenbosch, Private Bag X1, Tygerberg 7505, South Africa

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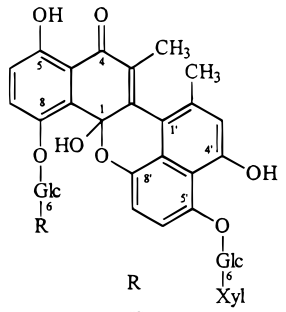
Our laboratory has engaged in the exploration of active antimicrobial principles present in chewing sticks commonly used by the African and Middle Eastern countries as a mechanical oral hygiene aid in place of tooth brushing. During this investigation, a methanol extract from the twigs of *Diospyros lycioides,* a Namibia tooth cleaning stick, demonstrated antimicrobial activity against common oral pathogens including *Streptococcus mutans* and *Porphyromonas gingivalis* (MICs 2.5 and 0.156 mg/mL). Subsequent fractionation and purification of this extract led to the identification of two novel binaphathalenone glycosides: 1′,2-binaphthalen-4-one-2′,3-dimethyl-1,8′-epoxy-1,4′,5,5′,8,8′-hexahydroxy-8-*O*-*β*-glucopyranosyl-5′-*O*-*β*-xylopyranosyl(1→6)-*β*-glucopyranoside (**1**) and 1′,2-binaphthalen-4-one-2′,3-dimethyl-1,8′-epoxy-1,4′,5,5′,8,8′-hexahydroxy-5′,8-di-*O*-*β*-xylopyranosyl(1→6)-*β*-glucopyranoside (**2**). Their structures were established using spectroscopic techniques. Examination of the antimicrobial activity of these two compounds revealed positive but only marginal growth inhibition against the test cariogenic pathogens, *S. sanguis* and *Streptococcus mutans*.

Mechanical aids, e.g., twigs and roots of plants, are still frequently employed by rural and even urban people in Africa for cleaning their teeth.[1] For this purpose, twigs from a variety of plants,[2] including those from *Salvadora persica*,[3] are used. A natural oral health survey in Namibia involving 2394 subjects in the age group 12 to 44 years revealed that approximately 20% of the participants examined in this study used *Diospyros lycioides* Desf. (Ebenaceae) twigs, a slender shrub that is widely distributed in this country, as tooth cleaning utensils.[4] During this survey, it was also noted that subjects in general had a relatively low caries rate.[5] It is known that *D. lycioides,* also known as Muthala,[6] has medicinal properties and contains some naphthoquinone constituents, including 7-methyljuglone, diospyrin, isodisopyrin, etc.[7,8] The search for plant-derived antimicrobial agents[9,10] prompted us to extract the twigs of this plant and further chemically analyze and test the compounds isolated for activity against oral bacteria.

The methanol extract of the twigs of *D. lycioides* demonstrated growth inhibitory activity against oral pathogens such as *Streptococcus sanguis* and *Porphyromonas gingivalis* at minimum inhibitory concentrations (MIC) of 2.5 and 0.156 mg/mL, respectively. This crude extract was subjected to Diaion HP-20 column chromatography and eluted with water followed by methanol. The fraction eluted with methanol was further chromatographed on silica gel and reversed-phase silica gel to afford glycosides **1** and **2**.

Glycoside **1** was obtained as a purple solid. It gave a yellow color with ferric chloride and a cherry-red color with sulfuric acid on TLC followed by heating. Glycoside **1** exhibited a quasi-molecular ion peak at *m*/*z* 847 $[M - H]^-$ in the negative ion FABMS; while in the positive ion FABMS, it displayed a quasi-molecular ion peak at *m*/*z* 871 $[M + Na]^+$. In conjunction with the analysis of the ^{13}C NMR spectrum, its molecular formula was deduced to be $C_{39}H_{44}O_{21}$. On acid hydrolysis, **1** afforded glucose and xylose in a ratio of 2:1 as sugar residues. The ^1H NMR spectrum of **1** displayed three anomeric proton signals at *δ* 4.29 (d, *J* = 7.1 Hz), 4.73 (d, *J* = 6.5 Hz), and 4.93 (d, *J* = 7.4 Hz), suggesting that all of the three sugars possessed the *β*-pyranosyl configurations. The presence of the three sugars was also supported by the anomeric carbon signals at *δ* 103.4, 103.6, and 103.8 in the ^{13}C NMR spectrum. The ^{13}C NMR spectrum of **1** due to aglycon moiety demonstrated 22 resonance signals (Table 1), among which there were two characteristic methyl signals at *δ* 14.1 and 20.4 in the upfield region and a carbonyl signal at *δ* 189.0 in the downfield region. The ^1H NMR spectrum due to aglycon moiety displayed two pairs of coupled aromatic doublets, one aromatic singlet, two methyl singlets, and three singlets corresponding to phenolic or hydroxyl protons (Table 1). These spectroscopic characteristics suggested that the aglycon of **1** was a



	R
1	H
2	Xyl

* To whom correspondence should be addressed. Tel.: (312) 335-1862. Fax: (312) 996-0943. E-mail: chriswu@uic.edu. This author has previously published under the name Christine D. Wu-Yuan.

[†] Current address: National Center for the Development of Natural Products, School of Pharmacy, University of Mississippi, Mississippi 38677.

[‡] University of Stellenbosch.

[§] University of Illinois at Chicago.

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Table 1. ^{13}C and ^{1}H NMR Data for Glycosides **1** and **2** in DMSO-d_6 (ppm)

	1		**2**	
C/H	δ_C	δ_H	δ_C	δ_H
aglycon				
1	89.9		89.9	
2	127.8		127.8	
3	145.4		145.3	
4	189.0		189.0	
5	156.3		156.3	
6	118.9	7.13 (d, J = 9.1 Hz)	119.3	7.14 (d, J = 9.2 Hz)
7	125.5	7.69 (d, J = 9.1 Hz)	125.1	7.87 (d, J = 9.2 Hz)
8	148.5		148.4	
9	128.0		127.9	
10	111.9		111.8	
11	14.1	1.82 (s)	14.1	1.82 (s)
1-OH		7.18 (s)		7.48 (s)
5-OH		12.57 (s)		12.58 (s)
1′	117.1		117.1	
2′	137.4		137.3	
3′	113.9	6.86 (s)	113.4	6.85 (s)
4′	154.6		154.6	
5′	148.7		148.7	
6′	112.9	7.54 (d, J = 8.4 Hz)	112.9	7.53 (d, J = 8.4 Hz)
7′	113.4	7.04 (d, J = 8.4 Hz)	113.4	7.06 (d, J = 8.4 Hz)
8′	143.2		143.2	
9′	122.7		122.7	
10′	113.1		113.0	
11′	20.4	2.30 (s)	20.4	2.29 (s)
4′-OH		9.68 (s)		
sugar				
Glc-1	103.4	4.73 (d, J = 6.5 Hz)	103.3	4.68 (d, J = 7.1 Hz)
2	73.8	3.23	73.6[a]	
3	76.0	3.24	75.8[b]	
4	69.7	3.35	69.8[c]	
5	77.4	3.41	76.6[d]	
6	60.6	3.80, 3.51	68.4[e]	4.07 (br d, J = 10.4 Hz)[h]
Xyl-1			104.1	4.30 (d, J = 7.2 Hz)[a]
2			73.3[a]	
3			76.6[d]	
4			69.4[f]	
5			65.5[g]	
Glc-1′	103.6	4.93 (d, J = 7.4 Hz)	103.6	4.92 (d, J = 7.2 Hz)
2′	73.4	3.40	73.4[a]	
3′	76.0	3.37	75.9[b]	
4′	69.9	3.15	69.9[c]	
5′	76.6	3.62	76.6[d]	
6′	68.2	4.07 (br d, J = 10.3 Hz), 3.58	68.2[e]	4.07 (br d, J = 10.4 Hz)[h]
Xyl-1′	103.8	4.29 (d, J = 7.1 Hz)	103.8	4.29 (d, J = 7.2 Hz)[a]
2′	73.4	3.04	73.4[a]	
3′	76.6	3.08	76.5[d]	
4′	69.5	3.29	69.5[f]	
5′	65.6	3.70 (dd, J = 11.1, 5.4 Hz), 2.99	65.6[g]	

[a–g] Signals in each column with the same superscript may be interchangeable. [h] Another proton of H-6 was not determined due to overlapping.

dimer of the 7-methyljuglone derivative.[11–14] Further information was obtained from 2D NMR experiments. HMQC of **1** established one-bond C−H connectivity, while $^{1}H-^{1}H$ COSY determined each spin-coupling system. In the HMBC spectrum of **1**, the *peri*-hydroxy proton signal at δ 12.57 (s) showed cross-peaks with the carbon signals at δ 156.3 (s), 118.9 (d), and 111.9 (s) (Figure 1). The methyl signal at δ 1.82 (s) displayed cross-peaks with the carbon signals at δ 189.0 (s) and 127.8 (s). The proton signal at δ 7.13 (d) correlated with three carbon signals at δ 148.5 (s), 156.3 (s), and 111.9 (s). The proton signal at δ 7.69 (d), which correlated with the signal at δ 7.13 (d) in the $^{1}H-^{1}H$ COSY, showed cross-peaks with four carbon signals at δ 156.3 (s), 148.5 (s), 128.0 (s), and 89.9 (s). The hemiketal hydroxy proton signal at δ 7.18 (s) displayed cross-peaks with the carbon signals at δ 89.9 (s) and 145.4 (s). The anomeric proton signal at δ 4.73 (d) showed a long-range

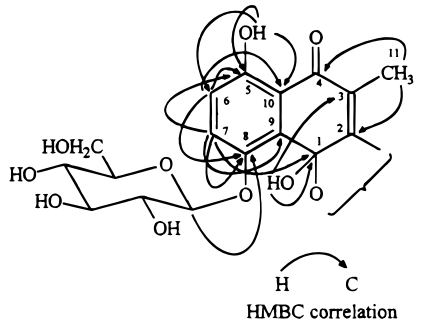

Figure 1. Partial structure **A** of **1** established by the HMBC experiment.

correlation with the carbon signal at δ 148.5 (s). This sugar was identified as a terminal glucose by a comprehensive analysis of $^{1}H-^{1}H$ COSY, HMQC, and HMBC spectra. The above evidence led to partial

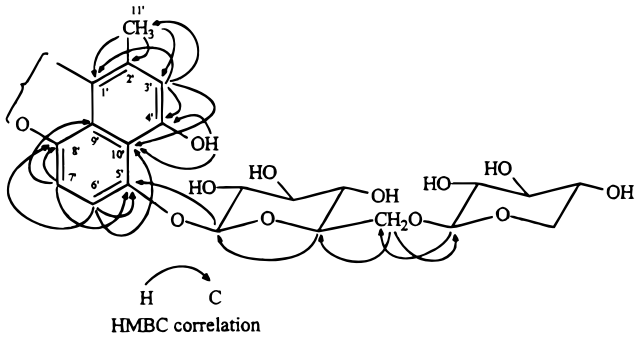

Figure 2. Partial structure **B** of **1** established by the HMBC experiment.

structure **A** of **1** as depicted in Figure 1. It was noted that the hemiketal proton showed a strong cross-peak with C-3. This kind of ^4J correlation was also observed between H-7 and C-1. In a similar manner, partial structure **B** of **1** was established as shown in Figure 2. In this partial structure, the linkage position of the terminal xylose to C-6 of the inner glucose was determined by the correlations through C/H-5 and C/H-6 of the glucose (Figure 2). It was observed that the glycosylation of the xylose to the hydroxy group of C-6 of the glucose resulted in downfield shifts for both C-6 (7.6 ppm) and one of H-6 (0.27 ppm) (Table 1). Therefore, the two partial structures (**A** and **B**) were linked through C-2 → C-1′ and C-1 → O → C-8′, and the structure of **1** was shown to be 1′,2-binaphthalen-4-one-2′,3-dimethyl-1,8′-epoxy-1,4′,5,5′,8,8′-hexahydroxy-8-*O*-*β*-glucopyranosyl-5′-*O*-*β*-xylopyranosyl(1→6)-*β*-glucopyranoside.

Glycoside **2** showed similar physical properties and TLC behavior as those of **1**. It produced a molecular ion peak at *m*/*z* 980 $[M]^-$ in the negative ion FABMS and a quasi-molecular ion peak at *m*/*z* 1003 $[M + Na]^+$ in the positive-ion FABMS, both being 132 mass units (corresponding to a pentose) more than those of **1**. Acid hydrolysis of **2** gave glucose and xylose in a ratio of 1:1 as sugar residues. The ^{13}C and ^1H NMR spectra of **2** due to aglycon moiety were superimposable on those of **1**, while the sugar moiety displayed a set of additional signals corresponding to an *β*-xylpyranosyl unit (Table 1). This indicated **2** was formed from **1** by glycosylation with a xylosyl unit. The linkage position of this xylose to C-6 of the inner glucose was confirmed by the glycosylation shifts ($\Delta\delta$ 7.8 ppm for C-6 and $\Delta\delta$ 0.27 ppm for one of H-6) (Table 1). Thus, the structure of **2** was formulated as 1′,2-binaphthalen-4-one-2′,3-dimethyl-1,8′-epoxy-1,4′,5,5′,8,8′-hexahydroxy-5′,8-di-*O*-*β*-xylopyranosyl(1→6)-*β*-glucopyranoside.

The characteristic of the two glycosides (**1** and **2**) was that their aglycons were formed by two 7-methyljuglone derivatives linked through a six-membered pyranol ring. This ring is considered to be unstable under acidic conditions. Upon spraying with sulfuric acid on TLC followed by heating, glycosides **1** and **2** showed cherry-red color, suggesting the formation of 1,4-binaphthoquinone. It is postulated that the 1,4-binaphthoquinone is the biogenetic precursor of glycosides **1** and **2**.

Subsequent effort was made to determine if glycosides **1** and **2** possessed growth inhibitory effect against common oral pathogenic bacteria. Upon testing, both glycosides inhibited growth of the Gram-positive cariogenic oral streptococci: *Streptococcus mutans* and *S. sanguis* with MICs both at 1 mg/mL. However, no inhibition was noted against the Gram-negative, anaerobic periodontal pathogens *P. gingivalis* and *Prevotella intermedia*. These glycosides appeared to preferentially inhibit the Gram-positive oral bacteria, and it is likely that this inhibition could have contributed to the lower caries rate observed in the chewing sticks users. Compared to the positive antimicrobial control, sanguinarine (MICs of 15 *μ*g/mL against the two bacteria),[10] the observed growth inhibitory activity of the two glycosides was significantly lower. Since the methanol extract was completely water soluble and contained a high level of polyphenols, it is believed that the latter could have contributed to the antimicrobial activity detected in the initial crude extract. Further isolation of additional active ingredients present in *D. lycioides* is currently underway.

Experimental Section

General Experimental Procedure. ^1H and ^{13}C NMR spectra were recorded in DMSO-d_6 with TMS as the internal standard, employing a Bruker WM-360 instrument operating at 360.14 and 90.08 MHz, respectively. $^1H-^1H$ COSY, HMQC, and HMBC (optimized for *J* value at 8 Hz) spectra were recorded with standard pulse sequences on a Bruker AMX-600 instrument. FABMS spectra were performed on a Finnigan MAT-90 instrument. Column chromatography: silica gel 60 (230−400 mesh, E. Merck) and reversed-phase silica gel (C_{18}, 40−60 *μ*m, Sigma). TLC: Merck aluminum-backed TLC sheets (Si gel F_{254}).

Plant Material. Authentic twigs of *D. lycioides* Desf. were collected in Namibia. A voucher specimen of this plant material was deposited at the Faculty of Dentistry, University of Stellenbosch, South Africa.

Extraction and Isolation. The dried twigs (100 g) were ground to a coarse powder and refluxed with MeOH (1 L × 3). Removal of the solvent yielded a MeOH extract (9.8 g) [MICs (mg/mL): *S. sanguis*, 2.5; *P. gingivalis*, 0.156]. The MeOH extract was dissolved in H_2O (50 mL). The solution was subjected to Diaion HP-20 eluted with H_2O and then MeOH. The H_2O eluate mainly contained sucrose. The MeOH eluate was concentrated to dryness to afford a purple residue (1.8 g) [MICs (mg/mL): *S. sanguis*, 1.25; *P. gingivalis*, 0.312]. This residue was chromatographed on silica gel gradually eluting with $CHCl_3-MeOH-H_2O$ (40:10:1 to 20:10:1) (a total of 3 L) to give fractions 1−132. Fractions 98−104 (60 mg) were chromatographed on reversed-phase silica gel (C_{18}) with 60% MeOH to yield **1** (10 mg). Fractions 116−118 (27 mg) were chromatographed on reversed-phase silica gel (C_{18}) with 50% MeOH to furnish **2** (6.5 mg).

Glycoside 1: purple solid; $[\alpha]^{25}_D \pm 0°$ (*c* 0.85, MeOH); FABMS (negative) *m*/*z* 847 $[M - H]^-$, 715 $[M - Xly (132)]^-$, 685 $[M - Glc (162)]^-$, 552 $[M - Xyl - Glc - H]^-$; FABMS (positive) *m*/*z* 871 $[M + Na]^+$, 709 $[M + Na - Glc]^+$; ^1H and ^{13}C NMR spectra, see Table 1.

Glycoside 2: purple solid, $[\alpha]^{25}_D \pm 0°$ (*c* 0.52, MeOH); FABMS (negative) *m*/*z* 980 $[M]^-$, 686 $[M - Xly (132) - Glc (162)]^-$; FABMS (positive) *m*/*z* 1003 $[M + Na]^+$, 709 $[M + Na - Xyl - Glc]^+$; ^1H and ^{13}C NMR spectra, see Table 1.

Acid Hydrolysis of 1 and 2. Each glycoside (1 mg) in 1 N HCl (0.2 mL) was heated at 90 °C for 1 h. After cooling, the reaction mixture was examined with TLC using $CHCl_3$−MeOH−AcOH−H_2O (14:6:2:1) as solvent system and 10% H_2SO_4 as color reagent. Glucose and xylose were detected with R_f values of 0.28 and 0.42, respectively, in a ratio of 2:1 for **1** and in a ratio of 1:1 for **2**.

Determination of Antimicrobial Activity against Oral Pathogens. Growth inhibitory effects of the two isolated novel compounds were tested against cariogenic oral streptococci including *S. mutans* Ingbritt and *S. sanguis* and the periodontal pathogen most frequently associated with periodontitis, *P. gingivalis and P. intermedia*. The procedures employed were as described previously.[9,10]

Sterile 96-well microtiter plates were used. Each well contained 5×10^5 colony-forming units (CFU)/mL of test bacteria, serially diluted test compounds, and the respective growth medium. Triplicate samples were performed for each test concentration. The controls included inoculated growth medium without test compounds. Sample blanks contained uninoculated medium only. All plates were incubated at 37 °C under appropriate atmospheric conditions with growth estimated spectrophotometrically (650 nm) after 48 h using a microtiter plate reader (Molecular Devices, Vmax Kinetics, Menlo Park, CA). The minimum inhibitory concentration (MIC) for each test bacterium was defined as the minimum concentration of test compound limiting turbidity to <0.05 absorbance at 650 nm. For positive control, the plant alkaloid sanguinarine with documented antimicrobial activity was used (Sigma Chemical Co., St. Louis, MO).

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References and Notes

(1) Addo-Yobo, C.; Williams, S. A.; Curzon, M. E. J. *Community Dental Health* **1991**, *8*, 155−162.
(2) Homer, K. A.; Manji, F.; Beighton, D. *Ach. Oral Biol.* **1990**, *35*, 421−424.
(3) Hardie, J.; Ahmed, K. *FDI World* July/Aug **1995**, 5−10.
(4) Schier, G. M. Oral Health in Namibia—A Pathfinder Survey. MSc. Thesis, Faculty of Dentistry, University of the Witwatersrand, Johannesburg, South Africa, 1993.
(5) Schier, M.; Cleaton-Jones, P. *Community Dent. Oral Epidemiol.* **1995**, *17*, 31−33.
(6) Arnold, H.-J.; Gulumian, M. *J. Enthnopharm*. **1984**, *12*, 35−74.
(7) Van Der Vijver, L. M.; Gerritsma, K. W. *Phytochemistry* **1974**, *13*, 2322−2323.
(8) Ferreira, M. A.; Costa, A. C.; Correia, A. *Plant Med. Phytother.* **1972**, *6*, 32.
(9) Cai, L.; Wu, C. D. *J. Nat. Prod.* **1996**, *59*, 987−990.
(10) Li, X.-C.; Cai, L.; Wu, C. D. *Phytochemistry* **1997**, *46*, 97−102.
(11) Sidhu, G. S.; Pardhasaradhi. *Tetrahedron Lett.* **1967**, 1313−1316.
(12) Fallas, A. L.; Thomson, R. H. *J. Chem. Soc. C* **1968**, 2279−2282.
(13) Ferreira, M. A.; Correia Alves, A.; Aurea Cruz Costa, M.; Isabel Paul, M. *Phytochemistry* **1977**, *16*, 117−120.
(14) Sankaram, A. V. B.; Narayana Reddy, V. V.; Marthandamurthi, M. *Phytochemistry* **1986**, *25*, 2867−2871.

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