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ARTICLE

Identification and Bioactivities of Resveratrol Oligomers and Flavonoids from *Carex folliculata* Seeds

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Plants of the *Carex* genus (Family: Cyperaceae) have attracted recent attention as potential food additives because they contain high levels of bioactive polyphenols commonly found in plant foods. Seven compounds, which included two resveratrol oligomers and five flavonoids, were isolated from seeds of *Carex folliculata* L. (northern long sedge), a forage prevalent in the northern United States. The compounds were identified by ^{1}H and ^{13}C nuclear magnetic resonance and mass spectrometry data. The resveratrol oligomers were pallidol (**1**), a resveratrol dimer reported to be present in levels equivalent to those of resveratrol in red wine, and kobophenol A (**2**), a resveratrol tetramer with a unique 2,3,4,5-tetraaryltetrahydrofuran skeleton. The flavonoids were isoorientin (**3**), luteolin (**4**), quercetin (**5**), 3-*O*-methylquercetin (**6**), and rutin (**7**). Compounds were evaluated for antioxidant activity in the diphenylpicrylhydrazyl (DPPH) radical scavenging assay; cytotoxicity activity against human colon (HCT116, HT29) and breast (MCF7, MDA-MB-231) tumor cell lines; and antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). The antioxidant activities of the flavonoids (**3**−**7**; IC_{50} values ranging from 50 to 200 μM) were comparable to that of ascorbic acid (IC_{50} = 60 μM) and superior to those of the resveratrol derivatives (**1** and **2**; IC_{50} > 1000 μM) and butylated hydroxytoluene (BHT; IC_{50} = 1500 μM), a commercial antioxidant. In the cytotoxicity and antibacterial bioassays, compounds **4** (IC_{50} for HCT116 = 45 μM) and **6** (IC_{50} for MRSA = 6.4 μM) were the most active, respectively. Therefore, given the wide availability and underutilization of *C. folliculata*, this forage may provide a source of bioactive compounds useful for nutraceutical purposes. Also, this is the first reported phytochemical investigation of *C. folliculata*.

KEYWORDS: *Carex folliculata*; stilbenes; flavonoids; nutraceuticals; bioactive

INTRODUCTION

Recently, there have been significant advances in scientific knowledge of the beneficial role of phytochemicals against several chronic human diseases mediated by oxidative stress such as cancer and heart disease. This has resulted in increasing consumer attention and sales of nutraceutical products that contain plant antioxidants, of which polyphenols constitute a large proportion. Among polyphenols, resveratrol (3,4′,5-trihydroxystilbene), a natural product present in grape and red wine and implicated in a wide range of biological effects, has received considerable attention. The biological properties of resveratrol range from antibacterial and antifungal effects to cardioprotective, neuroprotective, and anticancer effects (reviewed in ref *1*). Recent studies have also shown that resveratrol activates sirtuin deacetylases, thereby having positive effects on longevity and age-related deterioration (*2*, *3*). The implications of these findings are exciting considering that a large number of resveratrol derivatives, which may have similar or superior biological activities, are yet to be identified. Therefore, the search for novel resveratrol derivatives, obtained through synthesis or from natural product sources, have received keen scientific interest (*4*−*8*). As part of our laboratory's continuous effort to identify bioactive plant natural products useful for nutraceutical and/or phytopharmaceutical purposes, we initiated the current study. For this project, we focused our attention on a locally available (in the northern United States), abundant, underutilized, and previously uninvestigated plant belonging to a plant family, namely, Cyperaceae, known to produce stilbene derivatives. So far, resveratrol oligomers have been isolated mainly from five plant families: Cyperaceae, Dipterocarpaceae, Gnetaceae, Leguminosae, and Vitaceae (*8*).

In the Cyperaceae family, the genus *Carex* includes sedges that dominate wetlands, pastures, prairies, tundra, and the herb layer of temperate forests (*9*). Despite there being as many as 2000 *Carex* species worldwide, only a few members including *C. fedia*, *C. kobomugi*, *C. pumila*, *C. humilis*, *C. pendula*, and *C. distachya* have been previously investigated for their phytochemical constituents (*9*−*13*). This is unfortunate because *Carex* plants are characterized by the production of stilbene derivatives and other bioactive polyphenols including lignans and flavonoids (*9*−*13*). Recently, Fiorentino and co-workers reported that *C. distachya*,

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abundant in the Mediterranean *macchia*, should be considered as a potential food additive due to its high levels of antioxidant polyphenols commonly found in plant foods (*13*).

Because uninvestigated members of the *Carex* genus offer great potential for discovery of resveratrol oligomers and other polyphenols with rich chemical diversity, we investigated *C. folliculata* L. (northern long sedge), a forage widely prevalent in the northern United States. In the current study, we report the isolation, identification, and biological evaluation of seven compounds from the methanol extract of *C. folliculata* seeds. Two resveratrol oligomers and five flavonoids were isolated, and their antioxidant (ability to scavenge DPPH radicals), cytotoxicity (against human breast and colon tumor cell lines), and antibacterial (against methicillin-resistant *Staphylococcus aureus*, MRSA) activities are reported in this study. This is the first reported phytochemical investigation of *C. folliculata*.

MATERIALS AND METHODS

General Experimental Procedures. Nuclear magnetic resonance (NMR) spectra for compound **2** were obtained on a Bruker 300 MHz spectrometer, whereas NMR data for all other compounds were acquired on a Bruker 400 MHz Biospin spectrometer. Deuterated methanol (methanol-d_4) was used as solvent for all NMR experiments. Mass spectral (MS) data were carried out on a Q-Star Elite (Applied Biosystems MDS) mass spectrometer equipped with a Turbo Ionspray source and were obtained by direct infusion of pure compounds. High-performance liquid chromatography (HPLC) was performed on a Shimadzu LC-10AS pump with a photodiode array (PDA)−UV/vis detector and operated by Class VP version 4.2 software. Medium-pressure liquid chromatography (MPLC) was carried out on prepacked C_{18} columns connected to a DLC-10/11 isocratic liquid chromatography pump (D-Star Instruments, Manassas, VA) with a fixed-wavelength detector. All solvents were of ACS or HPLC grade and were obtained from Sigma-Aldrich through Wilkem Scientific (Pawcatuck, RI). Ascorbic acid, butylated hydroxytoluene (BHT), diphenylpicrylhydrazyl (DPPH) reagent, and 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfenyl)-2*H*-tetrazolium salt (MTS) were purchased from Sigma-Aldrich (St. Louis, MO).

Plant Material. *C. folliculata* L. aerial plant parts were collected in Westmoreland County, Pennsylvania, on September 15, 2007, and identified by Joseph A. Isaac. Voucher specimens (no. 19443) have been deposited at the Carnegie Museum Herbarium in Pittsburgh, PA.

Extraction, Isolation, and Structural Elucidation. Dried and ground *C. folliculata* seeds (64.0 g) was sequentially extracted with hexanes (2 × 400 mL), acetone (3 × 400 mL), and methanol (3 × 400 mL) to afford hexanes (2.4 g), acetone (3.2 g), and methanol (7.3 g) extracts, after solvent removal in vacuo, respectively. Because of our interest in identifying polyphenolic type compounds, we focused our isolation efforts on the most polar extract, that is, the methanol extract. The majority of the methanol extract (7.1 g) was subjected to a series of isolation methods that included column chromatography and MPLC with various stationary phases (XAD-16 Amberlite resin, silica gel, C_{18}, and Sephadex LH20), and preparative HPLC. First, the methanol extract was reconstituted in methanol/water (1:1, v/v), adsorbed onto an Amberlite XAD-16 resin column (45 × 3 cm), and eluted with water (3.5 L) followed by methanol (3.0 L). After solvent removal in vacuo, the resulting XAD-methanol eluate (3.8 g) was further purified by C_{18} MPLC (column, 37 × 5.5 cm) with a gradient solvent system of aqueous methanol (0.1% trifluoroacetic acid, TFA) to afford eight major fractions: A1−A8. Fraction A3 (300 mg) was further purified over a silica gel column with a gradient solvent system of methanol in chloroform to afford subfractions B1−B10. Subsequent silica gel column chromatography of subfraction B1 (50 mg) with a gradient solvent system of hexane in acetone afforded compound **1** (pallidol; 12 mg; off-white amorphous solid). Similarly, subfraction B2 was purified on a Sephadex LH-20 column (45 × 2.5 cm) with a gradient solvent system of methanol in water to afford compound **3** (isoorientin; 11 mg; yellow solid). All other isolates were obtained from fraction A4 (1.2 g), which was subjected to a C_{18} MPLC column (37 × 5.5 cm) with a gradient solvent system of aqueous methanol (0.1% TFA) to afford subfractions C1−C6. The C1−C4 subfractions were individually purified by preparative HPLC using a Waters Sunfire Prep C_{18} column (250 × 10 mm i.d., 5 μm; flow = 2 mL/min), with a gradient elution system of acetonitrile in water (0.1% TFA) to afford compounds **2** (kobophenol A; 22 mg; off-white amorphous solid), **4** (luteolin; 8.0 mg; yellow solid), **5** (quercetin, 10.0 mg; yellow solid), **6** (3-*O*-methylquercetin, 13.0 mg; yellow solid), and **7** (rutin, 20 mg; yellow solid). The structures of compounds **1**−**7** (shown in **Figure 1**) were established on the basis of NMR and MS data and by comparison with the literature (*14*−*19*).

NMR Data. The NMR data for the resveratrol oligomers, compounds **1** and **2**, are provided in parts **A** and **B** of **Table 1**, respectively. The NMR data for the flavonoids, compounds **3**−**7**, are provided in parts **A** and **B** of **Table 2**, respectively.

MS Data. Compound **1**: $[M - H]^-$ 453.1640 calcd for $C_{28}H_{22}O_6$. Compound **2**: $[M + H]^+$ 925.2100 calcd for $C_{56}H_{44}O_{13}$. Compound **3**: $[M - H]^-$ 447.1600 calcd for $C_{21}H_{20}O_{11}$. Compound **4**: $[M - H]^-$

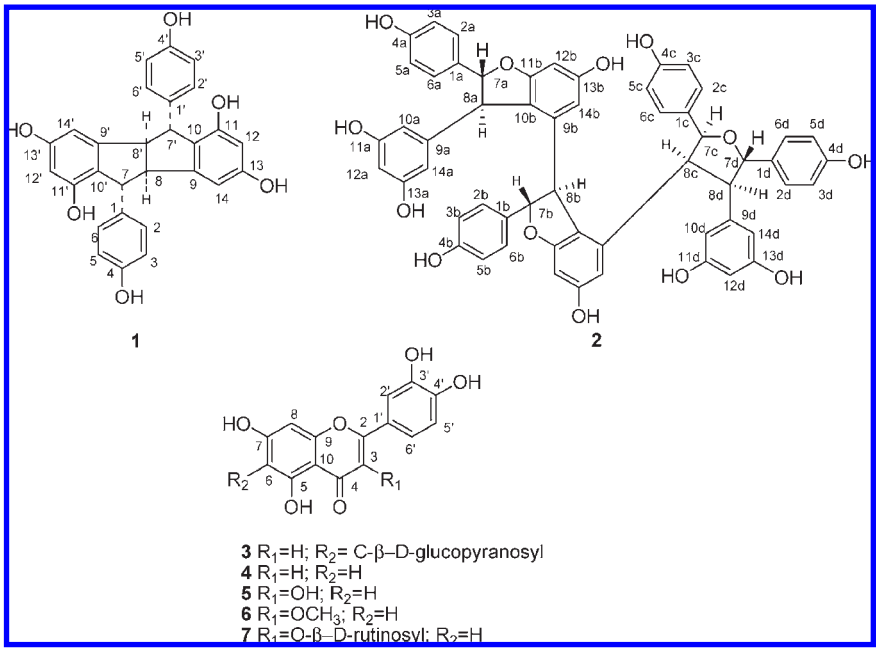

Figure 1. Compounds isolated and identified from *Carex folliculata*: pallidol (**1**), kobophenol A (**2**), isoorientin (**3**), luteolin (**4**), quercetin (**5**), 3-*O*-methylquercetin (**6**), rutin (**7**).

Table 1. NMR Spectroscopic Data for (**A**) Pallidol (**1**) and Kobophenol A (**2**)

(**A**) NMR Spectroscopic Data for **1**

	δ_C[a]	δ_H[a] (multiplicity, J_{HH} in Hz)
1,1′	138.6	
2,6,2′,6′	129.3	6.92 (d, 8.7)
3,5,3′,5′	116.1	6.66 (d, 8.7)
4,4′	156.3	
7,7′	54.9	4.48 (s)
8,8′	61.1	3.74 (s)
9,9′	150.9	
10,10′	123.93	
11,11′	155.7	
12,12′	102.6	6.11 (d, 2.1)
13,13′	159.5	
14,14′	103.1	6.54 (d, 2.1)

(**B**) NMR Spectroscopic Data for **2**

	δ_H[b]				δ_H[b] (multiplicity, J_{HH} in Hz)			
	a	b	c	d	a	b	c	d
1	133.6	134.0	131.9	139.5				
2,6[c]	126.7	127.4	127.8	128.9	7.29 (d, 8.7)	6.15 (d, 8.7)	6.43 (d, 8.7)	7.02 (d, 8.7)
3,5[c]	116.6	116.1	115.5	116.0	6.82 (d, 8.7)	6.42 (d, 8.7)	6.58 (d, 8.7)	6.72 (d, 8.7)
4	158.3	157.2	156.1	157.9				
7	92.3	94.3	85.6	85.9	5.47 (s)	5.00 (d, 3.9)	5.01 (d, 5.1)	5.10 (d, 10.5)
8	58.6	52.9	52.6	62.3	4.22 (s)	3.38 (d, 3.9)	3.22 (t, 5.1)	2.9 (dd, 10.5, 6.0)
9	147.8	144.8	134.1	136.6				
10	106.9	120.2	124.6	109.3	5.92 (brs)			5.66 (d, 2.1)
11	159.6	162.3	161.1	158.3				
12	102.1	96.3	95.8	103.4	5.90 (s)	6.49 (d, 2.1)	6.04 (d, 2.1)	5.93 (t, 2.1)
13	159.6	161.2	158.6	158.3				
14	106.9	108.8	110.9	109.3	5.92 (brs)	5.95 (d, 2.1)	6.37 (d, 2.1)	5.66 (d, 2.1)

[a] NMR data in MeOH-d_4 at 400 and 100 MHz for ^{1}H and ^{13}C, respectively. [b] NMR data in MeOH-d_4 at 300 and 75 MHz for ^{1}H and ^{13}C, respectively. [c] Equivalent.

285.0184 calcd for $C_{15}H_{10}O_6$. Compound **5**: $[M - H]^-$ 301.0189 calcd for $C_{15}H_{10}O_7$. Compound **6**: $[M - H]^-$ 315.0211 calcd for $C_{16}H_{12}O_7$. Compound **7**: $[M - H]^-$ 663.0114 calcd for $C_{27}H_{30}O_{16}$.

Antioxidant Assay. The antioxidant potentials of the compounds were determined on the basis of the ability to scavenge the 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical as previously reported (*20*). The DPPH radical scavenging activities of ascorbic acid (vitamin C) and the synthetic commercial antioxidant butylated hydroxytoluene (BHT) were also assayed as positive controls. The assay was conducted in a 96-well format using serial dilutions of 100 μL aliquots of test compounds (ranging from 2500 to 26 μg/mL), ascorbic acid (1000−10.4 μg/mL), and BHT (250000−250 μg/mL). Then DPPH (150 μL) was added to each well to give a final DPPH concentration of 137 μM. Absorbance was determined after 30 min at 515 nm, and the scavenging capacity (SC) was calculated as SC% = $[(A_0 - A_1/A_0)] \times 100$, where A_0 is the absorbance of the reagent blank and A_1 is the absorbance with test samples. The control contained all reagents except the compounds, and all tests were performed in triplicate. IC_{50} values denote the concentration of sample required to scavenge 50% DPPH free radicals. The positive controls, vitamin C and BHT, had IC_{50} values of 62 and 1500 μM, respectively, in this assay.

Antibacterial Assay. Antibacterial broth dilution assays were conducted using methicillin-resistant *S. aureus* (MRSA) (ATCC 43300, trypticase soy media) as previously reported (*21*). Briefly, MRSA was grown for 14 h in 10 mL tubes on a rotary shaker at 38 °C. Cultures were diluted with sterile medium to achieve an optical absorbance of 0.04−0.06 at 600 nm and then further diluted 10-fold before distribution into 96-well microtiter plates. Ten replicates of each of the test compounds were tested in dilution series, and results were measured after 18 h by measuring optical absorbance at 600 nm. Tetracycline was used as a positive standard and provided consistent IC_{50} values of 0.9 μM.

Cell Culture. All human tumor cell lines, MCF-7 and MDA-MB-231 (breast adenocarcinoma), HT-29 (colon adenocarcinoma), and HCT116 (colon carcinoma), were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and maintained in our laboratory at the University of Rhode Island. The cells were cultured in McCoy's 5A medium containing glutamine supplemented with 10% heat-inactivated fetal bovine serum, the antibiotic penicillin−streptomycin (10 mg/mL) (Invitrogen), and the addition of HEPES solution to control the pH of the media. All cell lines were incubated in a humidified environment at 37 °C in 5% CO_2 and maintained in the linear phase of growth.

Cytotoxicity Assay. The in vitro cytotoxicity assay was carried out as described previously (*22*). Briefly, the ability of the samples to inhibit the growth of a panel of human tumor cells was assessed by a tetrazolium-based colorimetric assay, which takes advantage of the metabolic conversion of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfenyl)-2*H*-tetrazolium, inner salt (MTS) to a reduced form that absorbs light at 490 nm. Cells were counted using a hemacytometer and were plated at 3750−10000 cells per well, depending on the cell line, in a 96-well format for 24 h prior to drug addition. Pure compounds and a positive standard, etoposide, were solubilized in DMSO by sonication. All test samples were diluted with media to the desired treatment concentration, and the final DMSO concentration per well did not exceed 0.3%. Control wells were also included on all plates. Following a 72 h drug incubation period at 37 °C with serially diluted test compounds, MTS, in combination with the electron coupling agent, phenazine methosulfate, was added to the wells. The incubation was continued for 3 h, and the absorbance of the medium was measured at 490 nm with a spectrophotometer (Spectramax M2, Molecular Devices, operated by Softmax-Pro v.4.6 software) to obtain the number of surviving cells relative to control populations. The results are expressed as the median cytotoxic concentrations (IC_{50} values) and were calculated from six-point dose−response curves using 4-fold serial dilutions. Each point on the curve was tested in triplicate. Data are expressed as mean ± SE for three replications. Etoposide provided consistent IC_{50} values of 6 μM (HT29,

Table 2. ^{13}C (**A**) and ^{1}H (**B**) NMR and Spectroscopic Data for Compounds **3**−**7**

(**A**) ^{13}C NMR Spectroscopic Data for **3**−**7**

	δ_C[a]				
	3	**4**	**5**	**6**	**7**
2	164.82	166.17	158.95	158.16	158.49
3	102.47	103.93	139.80	139.66	135.64
4	182.58	183.99	175.00	180.15	179.40
5	160.62	159.52	162.52	163.23	162.97
6	107.73	100.23	99.24	94.84	99.95
7	163.46	166.49	165.57	166.09	166.02
8	93.77	95.11	94.43	99.89	94.87
9	157.27	163.34	158.23	158.55	159.33
10	103.78	105.4	105.00	105.97	105.61
1′	122.73	123.74	124.15	123.02	123.57
2′	112.73	116.89	116.23	116.57	117.70
3′	145.62	151.15	146.22	146.12	145.83
4′	149.63	154.64	148.77	150.12	149.81
5′	115.73	114.24	115.99	116.57	116.05
6′	118.91	120.42	121.68	112.45	123.10
OCH_3				60.66	
1″	73.87				104.75
2″	71.16				75.93
3″	78.71				78.17
4″	70.38				71.38
5″	81.22				77.20
6″	61.47				68.55
1‴					102.42
2‴					72.10
3‴					72.23
4‴					73.93
5‴					69.71
6‴					17.90

(**B**) ^{1}H NMR Spectroscopic Data for **3**−**7**

	δ_H (multiplicity, J_{HH} in Hz)				
	3	**4**	**5**	**6**	**7**
3	6.55 (s)	6.45 (s)			
6		6.10 (d,2.1)	6.19 (d, 2.1)	6.21 (d, 2.1)	6.10 (d, 2.1)
8	6.45 (s)	6.40 (d,2.1)	6.40 (d, 2.1)	6.41 (d, 2.1)	6.29 (d, 2.1)
2′	7.38 (s)	7.74 (s)	7.74 (s)	7.63 (d, 2.1)	7.57 (d, 2.1)
5′	6.90 (d, 8.7)	6.89 (d, 8.7)	6.89 (d, 8.7)	6.92 (d, 8.7)	6.77 (d, 8.7)
6′	7.38 (d, 8.7)	7.64 (d,8.7)	7.64 (d, 8.7)	7.54 (dd, 8.7, 2.1)	7.54 (d, 8.7)
OCH_3				3.80 (s)	
1″	4.91 (d, 9.9)				5.0 (d, 7.2)
2″	4.21 (t, 9.3)				3.2−3.7 (m)
3″	3.50 (m)				3.2−3.7 (m)
4″	3.50 (m)				3.2−3.7 (m)
5″	3.50 (m)				3.2−3.7 (m)
6″	3.89 (dd, 12.0, 1.5), 3.77 (dd, 12.0, 5.1)				3.2−3.7 (m)
1‴					4.42 (s)
2‴					3.2−3.7 (m)
3‴					3.2−3.7 (m)
4‴					3.2−3.7 (m)
5‴					3.2−3.7 (m)
6‴					1.01 (d, 6.0)

[a] NMR data in MeOH-d_4 at 100 MHz. [b] NMR data in MeOH-d_4 at 400 MHz.

HCT116), 4 μM (MDA-MB-231), and 35 μM (MCF7) for the different human tumor cell lines.

RESULTS AND DISCUSSION

In the current study, seven compounds (structures shown in **Figure 1**) were isolated from a methanol seed extract of the forag, *C. folliculata*, also known as northern long sedge. The isolates were identified on the basis of NMR and MS data and by comparison to the literature (*14*−*19*) as resveratrol oligomers (**1** and **2**) and flavonoids (**3**−**7**).

Compounds **1** and **2** were identified as pallidol (a resveratrol dimer) and kobophenol A (a resveratrol tetramer), respectively. The NMR data for pallidol and kobophenol A are shown in parts **A** and **B** of **Table 1**, respectively. Pallidol, first isolated from *Cissus pallida*, is a natural constituent of grape and is reported to be present in red wine in equivalent levels to resveratrol (*23*−*25*).

Table 3. Biological Evaluation of Compounds **1**−**7** Showing 50% Inhibitory Concentrations (IC_{50} in Micromolar) in the Antioxidant (DPPH Radical Scavenging), Antibacterial (MRSA), and Human Cancer Cell [Breast (MCF7, MDA-MB231) and Colon (HT29, HCT116)] Cytotoxicity Assays[a]

	antioxidant[b,e]	antibacterial[c]	cytotoxicity[d,e]			
compd	DPPH	MRSA	MCF7	MDA-MB231	HT29	HCT116
1	>1500	NA	>100	>100	>100	>100
2	>1500	NA	>100	>100	>100	>100
3	57 ± 13	NA	>100	>100	>100	>100
4	161 ± 21	NA	>100	>100	91 ± 13	45 ± 5
5	70 ± 2	NA	>100	>100	>100	>100
6	188 ± 16	6.5 ± 1	97 ± 12	71 ± 5	>100	72 ± 12
7	71 ± 12	NA	>100	>100	>100	>100

[a] Values are means ± standard deviations. NA, not active. [b] Positive controls, ascorbic acid and BHT, had IC_{50} of 62 and 1500 μM, respectively. [c] Positive control, tetracycline, had IC_{50} of 0.9 μM. [d] Positive control, etoposide, had IC_{50} values of 6 μM (HT29, HCT116), 4 μM (MDA-MB-231), and 35 μM (MCF7). [e] Stated as >100 and >1500 μM for the cytotoxicty and antioxidant assays, respectively, when IC_{50} values exceed these concentrations.

Pallidol is formed by the dimerization of two resveratrol units, resulting in a symmetrical molecule as evident from its large number of equivalent signals in its NMR data (**Table 1A**) (*23*). Compound **2** was identified as kobophenol A, a resveratrol tetramer, first reported from *Carex kobomugi* (*15*). Although other resveratrol tetramers such as hopeaphenol have been previously reported in wines (*24*), the structure of kobophenol A is unique in that it contains a 2,3,4,5-tetraaryltetrahydrofuran skeleton not previously observed in resveratrol oligomers (*15*). Therefore, the evaluation of these molecules for their abilities to activate the SIRT-1 gene, as previously reported for resveratrol (*2*, *3*), is of great interest and will be investigated in future planned studies in our laboratory.

Compounds **3**−**7** were identified as the flavonoids isoorientin (**3**) (i.e., luteolin-6-*C*-β-D-glucopyranoside), luteolin (**4**), quercetin (**5**), 3-*O*-methylquercetin (**6**), and rutin (**7**) (NMR data shown in **Table 2**). Whereas flavone *C*-glucosides are not as common as flavone *O*-glucosides in plants, the identification of isoorientin (**3**) in the *C. folliculata* forage is not unusual because this compound has been previously reported in other grass plants, namely, blue grass (*Poa ampla*) (*26*).

Compounds **1**−**7** were evaluated for antioxidant activity in the DPPH radical scavenging assay, cytotoxicity activity against human colon (HCT116, HT29) and breast (MCF7, MDA-MB-231) tumor cell lines, and antibacterial activity against MRSA (see **Table 3**).

The antioxidant activities of the flavonoids (**3**−**7**; IC_{50} values ranging from 57 to 188 μM) were comparable to that of ascorbic acid (IC_{50} = 60 μM) and superior to those of the resveratrol derivatives (**1**−**2**; IC_{50} > 1500 μM) and butylated hydroxytoluene (BHT; IC_{50} = 1500 μM), a commercial antioxidant. The most active compound in the antioxidant assay was isoorientin (**3**; IC_{50} = 57 μM). Previous studies have shown that isoorientin-6″-*O*-glucoside is a very potent water-soluble antioxidant compared to several antioxidants including BHT and Trolox (*27*). Indeed, in the current study, we observed that the antioxidant activity of isoorientin (**3**) was superior to that of its corresponding aglycon, luteolin (**4**) (IC_{50} = 57 vs 161 μM). The observed increase in antioxidant activity with concomitant increase in degrees of glycosylation (isoorientin-6-*O*-glucoside > isorientin > luteolin) could probably be due to increasing water solubility in this "aqueous-based" antioxidant assay. Increasing degrees of glycosylation would increase the polarity and, therefore, the solubility of these compounds in water. Given the wide number of in vitro antioxidant assays currently utilized by laboratories, a different trend in a "lipophilic-based" antioxidant assay is very possible (*28*, *29*). It is noteworthy that recent studies have proposed that pallidol (**1**), a potent and selective singlet oxygen quencher, may play an important role in singlet-oxygen-mediated diseases (*30*). However, the results obtained from in vitro assays should be taken in context with respect to the biological effects exerted by these compounds in vivo, where factors such as their bioavailability and metabolism have to be considered. Nevertheless, because the level of pallidol in red wine is relatively low, that is, 0.5−4.8 mg/L (*24*, *25*, *30*), the isolation of this compound from an inexpensive natural source such as *C. folliculata* forage is of great commercial interest to the nutraceutical industry.

In the cytotoxicity assay, the breast adenocarcinoma cancer cells were most sensitive to compound **6** (MCF7, IC_{50} = 97 μM; MDA-MB-231, IC_{50} = 71 μM). However, the colon adenocarcinoma (HT29) and colon carcinoma (HCT116) cells were most sensitive to compound **4**, with IC_{50} values of 91 and 45 μM, respectively. In the antibacterial assay, only compound **6** inhibited MRSA bacterial growth, with an IC_{50} value of 6.5 μM. Although we did not observe any antibiotic effects of compound **2** against MRSA, a prior study reported moderate antibiotic properties of this isolate against *S. aureus* (*15*).

In summary, seven polyphenols (**1**−**7**), which included two resveratrol oligomers and five flavonoids, commonly present in plant foods, were isolated from seeds of *C. folliculata*. Seeds of many plant species generally contain, or have the ability to produce, compounds with a wide range of biological properties, and although these compounds may also be present in other parts of a plant, they are often found at higher concentrations in seeds (*31*). Therefore, given the wide availability and underutilization of *C. folliculata* in the northern United States, the seeds of this forage may provide a nutraceutical source of bioactive compounds.

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LITERATURE CITED

(1) *Resveratrol in Health and Disease*;Aggarwal, B. B., Shishodia, S., Eds.; CRC Press, Taylor and Francis Group: Boca Raton, FL, 2006.
(2) Howitz, K. T.; Bitterman, K. J.; Cohen, H. Y.; Lamming, D. W.; Lavu, S.; Wood, J. G.; Zipkin, R. E.; Chung, P.; Kisielewski, A.; Zhang, L. L.; Scherer, B.; Sinclair, D. A. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature* **2003**, *425*, 191–196.
(3) Baur, J. A.; Sinclair, D. A. Therapeutic potential of resveratrol: the in vivo evidence. *Nat. Rev. Drug Discov.* **2006**, *5*, 493–506.
(4) Joseph, J. J.; Fisher, D. R.; Cheng, V.; Rimando, A. M.; Shukitt-Hale, B. Cellular and behavioral effects of stilbene resveratrol analogues: implications for reducing the deleterious effects of aging. *J. Agric. Food Chem.* **2008**, *56*, 10544–10551.
(5) Snyder, S. A.; Breazzano, S. P.; Ross, A. G.; Lin, Y.; Zografos, A. L. Total synthesis of diverse carbogenic complexity within the resveratrol class from a common building block. *J. Am. Chem. Soc.* **2009**, *131*, 1753–1765.
(6) Yang, H.; Baur, J. A.; Chen, A.; Miller, C.; Adams, J. K.; Kisielewski, A.; Howitz, K. T.; Zipkin, R. E.; Sinclair, D. A. Design and synthesis of compounds that extend yeast replicative lifespan. *Aging Cell* **2007**, *6*, 35–43.
(7) Burns, J.; Yokota, T.; Ashihara, H.; Lean, M. E. J.; Crozier, A. Plant foods and herbal sources of resveratrol. *J. Agric. Food Chem.* **2002**, *50*, 3337–3340.
(8) Sotheeswaran, S.; Pasupathy, P. Distribution of resveratrol oligomers in plants. *Phytochemistry* **1993**, *32*, 1083–1092.
(9) Fiorentino, A.; D'Abrosca, B.; Pacifico, S.; Izzo, A.; Letizia, M.; Esposito, A.; Monaco, P. Potential allelopatic effects of stilbenoids

and flavonoids from leaves of *Carex distachya* Desf. *Biochem. Syst. Ecol.* **2008**, *36*, 691–698.
(10) Meng, Y.; Bourne, P.C.; Whiting, P.; Sik, V.; Dinan, L. Identification and ecdysteroid antagonist activity of three oligostilbenes from the seeds of *Carex pendula* (Cyperaceae). *Phytochemistry* **2001**, *57*, 393–400.
(11) Kurihara, H.; Kawabata, J.; Ichikawa, S.; Mizutani, J. (−)-ε-Viniferin and related oligostilbenes from *Carex pumila* Thunb. (Cyperaceae). *Agric. Biol. Chem.* **1990**, *54*, 1097–1099.
(12) Suzuki, K.; Shimizu, T.; Kawabata, J.; Mizutani, J. New 3,5,4′-trihydroxystilbene (resveratrol) oligomers from *Carex fedia* Nees var. *miyabei* (Franchet) T. Koyama (Cyperaceae). *Agric. Biol. Chem.* **1987**, *51*, 1003–1008.
(13) Fiorentino, A.; Ricci, A.; D'Abrosca, B.; Pacifico, S.; Golino, A.; Letizia, M.; Piccolella, S.; Monaco, P. Potential food additives from *Carex distachya* roots: identification and in vitro antioxidant properties. *J. Agric. Food Chem.* **2008**, *56*, 8218–8225.
(14) Snyder, S. A.; Breazzano, S. P.; Ross, A. G.; Lin, Y.; Zografos, A. L. Total synthesis of diverse carbogenic complexity within the resveratrol class from a common building block. *J. Am. Chem. Soc.* **2009**, *131*, 1753–1765.
(15) Kawabata, J.; Ichikawa, S.; Kurihara, H.; Mizutani, J. Kobophenol A, a unique tetrastilbene from *Carex kobomugi* Ohwi (Cyperaceae). *Tetrahedron Lett.* **1989**, *30*, 3785–3788.
(16) Leitao, A. G.; Monache, F. A. 2″-*O*-Caffeoylorientin from *Vitex polygama*. *Phytochemistry* **1998**, *49*, 2167–2169.
(17) Markham, K. R.; Chari, V. M.; Mabry, T. J. Carbon-13 NMR spectroscopy of flavonoids. In *The Flavonoids: Advances in Research*; Harborne, J. B., Mabry, T. J., Eds.; Chapman and Hall: New York, 1982; pp 19−134.
(18) Agrawal, P. K.; Bansal, M. C. Flavonoid glycosides. In *Studies in Organic Chemistry 39: Carbon-13 NMR of Flavonoids*; Agrawal, P. K., Ed.; Elsevier: New York, 1989; pp 283−364.
(19) Shen, C. C.; Chang, Y. S.; Ho, L. K. Nuclear magnetic resonance studies of 5,7 dihydroxyflavonoids. *Phytochemistry* **1993**, *34*, 843–845.
(20) Nanjo, F.; Goto, K.; Seto, R.; Suzuki, M.; Sakai, M.; Hara, Y. Scavenging effects of tea catechins and their derivatives on 1,1-diphenyl-2-picrylhydrazyl radical. *Free Radical Biol. Med.* **1996**, *21*, 895–902.
(21) Socha, A. M.; Garcia, D.; Sheffer, R.; Rowley, D. C. Antibiotic bisanthraquinones produced by a streptomycete isolated from a cyanobacterium associated with *Ecteinascidia turbinata*. *J. Nat. Prod.* **2006**, *69*, 1070–1073.
(22) Cory, A. H.; Owen, T. C.; Barltrop, J. A.; Cory, J. G. Use of an aqueous soluble tetrazolium/formazan assay for cell growth assays in culture. *Cancer Commun.* **1991**, *3*, 207–212.
(23) Khan, M. A.; Nabi, S. G.; Prakash, S.; Zaman, A. Pallidol, a resveratrol dimer from *Cissus pallida*. *Phytochemistry* **1986**, *25*, 1945–1948.
(24) Guebailia, H.; Chira, K.; Richard, T.; Mabrouk, T.; Furiga, A.; Vitrac, X.; Monti, J. P.; Delaunay, J. C.; Mérillon, J. M. Hopeaphenol: the first resveratrol tetramer in wines from North Africa. *J. Agric. Food Chem.* **2006**, *54*, 9559–9564.
(25) Vitrac, X.; Monti, J. P.; Vercauteren, J.; Deffieux, C.; Mérillon, J. M. Direct liquid chromatographic analysis of resveratrol derivatives and flavanonols in wines with absorbance and fluorescence detection. *Anal. Chim. Acta* **2002**, *458*, 103–110.
(26) Ju, Y.; Sacalis, J. N.; Still, C. C. Bioactive flavonoids from endophyte-infected blue grass (*Poa ampla*). *J. Agric. Food Chem.* **1998**, *46*, 3785–3788.
(27) Ko, F. G.; Chu, C. C.; Lin, C. N.; Chang, C. C.; Teng, C.-M. Isoorientin-6″-*O*-glucoside, a water soluble antioxidant isolated from *Gentiana arisanensis*. *Biochim. Biophys. Acta* **1998**, *1389*, 81–90.
(28) Prior, R. L.; Wu, X.; Schaich, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* **2005**, *53*, 4290–4302.
(29) Huang, D.; Ou, B.; Prior, R. L. The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.* **2005**, *53*, 1841–1856.
(30) He, S.; Jiang, L.; Wu, B.; Pan, Y.; Sun, C. Pallidol, a resveratrol dimer from red wine, is a selective singlet oxygen quencher. *Biochem. Biophys. Res. Commun.* **2009**, *379*, 283–287.
(31) Powell, P. G. Plant seeds as sources of potential industrial chemicals, pharmaceuticals, and pest control agents. *J. Nat. Prod.* **2009**, *72*, 516–523.

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