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Identification of Radical Scavengers in Sweet Grass (*Hierochloe odorata*)

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Extracts from aerial parts of sweet grass (*Hierochloe odorata*) were active DPPH free radical scavengers. The active compounds were detected in extract fractions using HPLC with on-line radical scavenging detection. After multistep fractionation of the extract, two new natural products possessing radical scavenging activity were isolated, and their structures were elucidated by NMR and MS. They were identified as 5,8-dihydroxybenzopyranone and 5-hydroxy-8-*O*-β-D-glucopyranosyl-benzopyranone. Activities of the compounds isolated were tested by DPPH and ABTS free radical scavenging assays, and compared with the known natural antioxidant rosmarinic acid and Trolox.

KEYWORDS: Sweet grass; *Hierochloe odorata*; fractionation; identification; radical scavengers

INTRODUCTION

Autoxidation of lipids, which can be induced by light, temperature, oxygen, and other factors, significantly decreases the quality of fat-containing foods. Consuming such foods is associated with aging, heart disease, stroke, and cancer. Therefore, antioxidants are widely used in foods, and also in cosmetics and pharmaceuticals (*1*). Since the 1980s there has been an increased interest in research and application of natural antioxidants instead of synthetic ones, caused by a consumer demand for natural food additives. Additionally, the burden of proof of safety may be less rigorous than that required for synthetic antioxidants (*2*, *3*). The antioxidant activity of many plants has been investigated (*4*−*8*), however, to date, only rosemary and sage extracts are commercially available as flavorless, odorless, and colorless antioxidant extracts.

Sweet grass (*Hierochloe odorata* L.) belongs to the family Graminaeae. The root and the aerial parts of the herb possess a sweet smell. Sweet grass is a hardy aromatic perennial grass normally found growing in rich, moist soil from Alaska to Newfoundland in full sun, but is also native to northern Europe. To our knowledge the publications on sweet grass properties and chemical composition are few. Only volatile compounds of this herb have been investigated (*9*, *10*). No reports were found on the antioxidative activity of sweet grass. However, preliminary screening results of sweet grass showed that extracts of this herb retard lipid oxidation (*11*). The aim of this study was to identify the compounds responsible for the radical scavenging activity of sweet grass extracts.

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MATERIALS AND METHODS

Chemicals. The following solvents were used for the extraction and fractionation: methanol, hexane, *tert*-butyl methyl ether, and butanol. All solvents were distilled prior to use. Solvents used for preparative chromatography and antioxidation activity testing were of analytical grade (Sigma Chemical, St. Louis, MO). For HPLC separations solvents of HPLC grade (Lab-Scan Analytical Sciences, Dublin, Ireland) were used. The following reagents were used in the antioxidant activity experiments: 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) (95%, Sigma-Aldrich Chemie, Steinheim, Germany), rosmarinic acid (Extrasynthese, Genay, France), 2,6-*di*-*tert*-butyl-hydroxytoluene (BHT) (Sigma-Aldrich Chemie, Steinheim, Germany), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diamonium salt (ABTS) (Fluka Chemie, Buchs, Switzerland), and Trolox 97% (Sigma-Aldrich Chemie, Steinheim, Germany). Deuterated methanol, deuterated chloroform, and deuterated dimethyl sulfoxide (Acros Organics, Geel, Belgium) were used to prepare solutions of compounds for NMR analysis.

Preparation of Plant Extracts. Aerial parts of *H. odorata* were obtained from the collection of the Kaunas Botanical Garden in 1998, air-dried in a Vasara ventilated oven (Utenos krosnys, Utena, Lithuania) at 30 °C for about 48 h, and ground before use. Dried and ground plant material (50 g) was extracted (2 × 1 L) with methanol/water/acetic acid (80:20:1) at room temperature for 24 h. Solvent and plant material were constantly mixed on a IKAMAG RTC basic magnetic stirrer (IKA Labortechnik, Staufen, Germany). The extract obtained was concentrated in a rotary evaporator at 40 °C to about 150−200 mL. The solution was diluted to 500 mL with ultrapure water and then successively extracted with several 100-mL vol of hexane, *tert*-butyl methyl ether, and finally butanol. Total amounts of 500−600 mL of each solvent were used. The remaining aqueous phase was freeze-dried. Another 40 g of dried and ground plant material was extracted with 1.5 L of acetone in a Soxhlet apparatus for 4 h and treated in the same way as the methanol/water/acetic acid extract. Yields of the fractions are given in **Table 1**.

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Table 1. Yields of Sweet Grass Extracts and Fractions and Their DPPH Scavenging %

extraction method	fraction	yield, %	DPPH scavenging %
MeOH 80%	crude extract	16.9	52.2 ± 0.8
water 20%	hexane	1.9	13.5 ± 0.5
acetic acid 1%	*tert*-butyl methyl ether	1.2	86.9 ± 0.1
	butanol	6.1	34.2 ± 0.3
	water	7.7	5.7 ± 1.1
acetone	crude extract	9.3	84.1 ± 0.1
oleoresin	hexane	1.9	22.3 ± 0.8
	tert-butyl methyl ether	2.1	81.8 ± 0.1
	butanol	1.6	63.5 ± 0.8
	water	0.5	19.7 ± 0.2

DPPH Assay. Radical scavenging activity of sweet grass extracts, BHT, and rosmarinic acid, against the stable radical DPPH$^{\bullet}$ was measured using the method of Von Gadow et al. (*12*) modified as described below. Methanolic solutions of DPPH$^{\bullet}$ (10^{-4} M) were mixed in a 1-cm path length disposable plastic half-micro cuvette (Greiner Labortech, Alphen a/d Rijn, The Netherlands) with sweet grass extracts and reference compounds BHT and rosmarinic acid in such a way that the final mass ratio of the extract to DPPH$^{\bullet}$ was 3 to 1. The samples were kept 15 min in the dark at room temperature, and the decrease of absorbance at 515 nm was measured against methanol using a Lambda 18 spectrophotometer (PerkinElmer, Ueberlingen, Germany). The absorbance of a blank sample containing the same amount of methanol and DPPH$^{\bullet}$ solution was prepared and measured daily. DPPH$^{\bullet}$ solution was freshly prepared daily and kept in the dark at 4 °C between the measurements. All determinations were performed in triplicate. The radical scavenging activity of the tested samples, expressed as % inhibition, was calculated by the following formula (*13*):

$$\% \text{ Inhibition} = [(A_B - A_A)/A_B] \times 100 \quad (1)$$

where A_B is the absorbance of the blank sample ($t = 0$), and A_A is the absorbance of sample with antioxidant after 15 min.

The activities of isolated pure compounds were measured using the method described above except that the concentrations of compounds tested and DPPH were taken on a molar basis, at the ratios needed to establish the concentration needed to scavenge 50% of the DPPH (*14*).

ABTS Decolorization Assay. ABTS$^{\bullet+}$ radical cation was produced by reacting ABTS with potassium persulfate (*15*). To prepare the stock solution, ABTS was dissolved at a 2 mM concentration in 50 mL of phosphate buffered saline (PBS) prepared from 8.18 g of NaCl, 0.27 g of KH_2PO_4, 1.42 g of Na_2HPO_4, and 0.15 g of KCl dissolved in 1 L of ultrapure water. If the pH was lower than 7.4, it was adjusted with NaOH. A 70 mM $K_2S_4O_8$ solution in ultrapure water was prepared. ABTS radical cation was produced by reacting 50 mL of ABTS stock solution with 200 μL of $K_2S_4O_8$ solution and allowing the mixture to stand in the dark at room temperature for 16−17 h before use. The radical was stable in this form for more than 2 days when stored in the dark at room temperature. For the study of antioxidant compounds, the ABTS$^{\bullet+}$ solution was diluted with PBS to an absorbance of 0.800 ± 0.030 AU at 734 nm. Stock solutions of the compounds in methanol were diluted with 10% methanol in PBS such that after introduction of a 10-μL aliquot of each dilution into the assay, they produced 10−80% inhibition of the blank absorbance.

After addition of 990 μL of diluted ABTS$^{\bullet+}$ solution ($A_{734\text{ nm}} = 0.800 \pm 0.030$) to 10 μL of antioxidant compounds or Trolox standards (final concentration 0−20 μM) in ethanol or PBS, the absorbance was read at ambient temperature exactly 1 and 6 min after the initial mixing. Appropriate solvent blanks were run in each assay. All determinations were carried out in triplicate. The percentage decrease of the absorbance at 734 nm was calculated and plotted as a function of the concentration of the antioxidants and of Trolox for the standard reference data.

To calculate the Trolox equivalent antioxidant coefficient (TEAC), the slope of the plot of the percentage inhibition of absorbance vs concentration for the antioxidant was divided by the slope of the plot of Trolox. This gives the TEAC at the specific time point (*15*).

HPLC−DPPH Conditions and Instrumentation. The on-line DPPH scavenging tests were performed using the method developed by Koleva et al. (*16*) and modified by Dapkevicius et al. (*17*) on an HPLC system equipped with a Waters 600E multisolvent delivery system (Millipore Corp., Waters Chromatography Division, Milford, MA), and an autosampling injector model 231 (Gilson Medical Electronics, Middleton, WI). The linear binary gradient was formed at a constant flow rate of 0.8 mL/min. Solvent A was a 20% methanol solution in water, and solvent B was 100% methanol. Initial isocratic conditions of 100% solvent A for 8 min was followed by an increase to 100% solvent B during 17 min, then isocratic conditions for 17 min. Finally, the gradient was returned to its initial conditions in 3 min and the column was equilibrated during 5 min. Separation of compounds was carried out on a 25 cm × 0.46 cm i.d. end-capped Alltima C18 analytical column (Alltech Associates, Deerfield, IL). Compounds eluted from the column were detected with a Waters 990 series photodiode array detector (Millipore) over the range 210−450 nm. Data were processed with Waters software, version LCA-6.22a. After the separation and detection, a 10^{-4} M solution of DPPH in methanol was added with a 45-mL laboratory-made syringe pump (Free University, Amsterdam, The Netherlands) at a flow rate of 0.70 mL/min. The mixture was continuously introduced into a 15-m reaction coil, and the decrease in absorbance of a DPPH solution was measured at 517 nm with a 759A model absorbance detector (Applied Biosystems, Foster City, CA) equipped with a tungsten lamp.

Isolation of Active Compounds. Fractionation conditions were determined using silica gel 60 F_{254} TLC plates (5 × 10 cm) (Merck, Darmstadt, Germany). The fractionation of the *tert*-butyl methyl ether fraction (0.4 g) was performed with a 50-g silica gel column (40−63 μm, Fluka Chemie, Buchs, Switzerland) with ethyl acetate/hexane (1:1). A total of 100 fractions (10 mL each) were collected. Radical scavenging activity was determined by spotting fractions on a TLC plate and then spraying the TLC plate with 0.2% DPPH solution in methanol. The active fractions were 9 to 19. Active fractions were then checked for purity on TLC. Fractions 11 to 19 were found to contain a single compound and they were combined and evaporated to dryness with a rotary evaporator. A yellow crystalline material (141 mg), **1**, (mp 216 °C) was obtained. The yield of **1** was 0.44% based on the dry plant material.

A 0.8-g butanol fraction was separated with chloroform/methanol/water (60:22:4) on an 80-g silica gel column, and 85 fractions were collected. Fractions 4−6 and 13−17 showed activity in the DPPH test. TLC showed that in fractions 4−6 the same active compound **1** was present that was previously isolated from the *tert*-butyl methyl ether fraction. The radical scavenging fractions 14−16 were combined, and the solvent was evaporated with a rotary evaporator. The material was then dissolved in methanol and left overnight in a refrigerator. The white crystals (mp 197 °C) that were formed were separated from the solvent and dried, and 62 mg of compound **2** was obtained (yield of 0.47% based on the dry plant material).

Instruments. ^{1}H NMR spectra were recorded on Bruker DPX 400 or Bruker AC-E 200 spectrometers (Bruker, Fällanden, Switzerland). ^{13}C spectra were recorded on a Bruker DPX 400 operated at 100 MHz. DEPT spectra and 2D experiments (COLOC and HMBC) and deuteration experiments were performed on a Bruker DPX 400.

NMR spectra of compound **1** were recorded in a mixture of deuterated chloroform and deuterated methanol (4:1). For the deuteration experiment 4 drops of nondeuterated methanol were added, and the ^{13}C NMR spectrum was recorded.

NMR spectra of compound **2** were recorded in deuterated DMSO. NMR assignments are shown in **Table 2**.

Mass spectra and accurate mass measurements were recorded on a Finnigan/MAT95 MS analyzer (Thermo Finnigan MAT, Bremen, Germany) in the EI mode.

UV spectra were recorded on a Lambda 18 spectrophotometer (PerkinElmer, Ueberlingen, Germany), and IR spectra were recorded on a PerkinElmer 1725 X FTIR spectrometer. Optical rotation measurements were performed in a 10-cm 1-mL measuring cell on a PerkinElmer 241 polarimeter using a sodium lamp at 589 nm.

Melting points of compounds were measured on a Buchi 510 apparatus (Buchi, Flawil, Switzerland).

Table 2. ^{1}H and ^{13}C NMR Data of the Compounds Isolated from Sweet Grass

compound	solvent	molecular formula	^{1}H NMR, δ (ppm)	^{13}C NMR, δ (ppm)
1	chloroform[a]–methanol	$C_9H_6O_4$	H-3, 6.24 (d, J = 9.7 Hz) H-4, 8.10 (d, J = 9.7 Hz) H-6, 6.54 (d, J = 8.8 Hz) H-7, 6.92 (d, J = 8.8 Hz)	C-2, 162.3 C-3, 113.3 C-4, 141.1 C-5, 147.4 C-6, 110.1 C-7, 120.1 C-8, 137.0 C-9, 142.7 C-10, 109.6
2	DMSO[b]	$C_{15}H_{16}O_9$	H-3 6.33 (d, J = 9.7 Hz) H-4, 8.18 (d, J = 9.7 Hz) H-6, 6.61 (d, J = 8.9 Hz) H-7, 7.34 (d, J = 8.9 Hz) H-1′, 4.89 (d, J = 7.7 Hz) H-2′+3′+5′, 3.28 (m) H-4′, 3.16 (dd, J = 8.7 and 8.7 Hz) H-6a′, 3.43 (m) H-6b′, 3.46 (m)	C-2, 159.6 C-3, 108.7 C-4, 139.4 C-5, 148.6 C-6, 113.6 C-7, 119.3 C-8, 136.5 C-9, 143.6 C-10, 113.6 C-1′, 100.9 C-2′, 73.1 C-3′, 76.6 C-4′, 69.6 C-5′, 76.9 C-6′, 61.4

[a] In ^{1}H, spectra calibrated on the residual $CHCl_3$ signal at 7.26 ppm; in ^{13}C, spectra calibrated at 77.2 ppm. [b] In ^{1}H, spectra calibrated on the residual DMSO signal at 2.5 ppm; in ^{13}C, spectra calibrated at 39.5 ppm.

Spectral Data 1. UV (MeOH) λ_{max} 267, 305 nm and 363 nm. IR (KBr) 3397, 3221, 1690 (C═O), 1621, 1581, 1509, 1460, 1189, and 1037 cm^{-1}. EIMS spectrum (70 eV) *m*/*z* 178 (M^+, 100%), 150 (11), 122 (20), 94 (19), 66 (10). Accurate mass measurements see text. ^{1}H and ^{13}C NMR spectra see **Table 2**.

Spectral Data 2. UV (MeOH) λ_{max} 260, 301, and 349 nm. IR (KBr) 3332, 2945, 2833, 1450, 1115, and 1027 cm^{-1}. FDMS *m*/*z* 341 (79), 340 (M^+, 81%) 178 (74), 177 (31), 163 (13). $[\alpha]_D^{20} = -63°$ (c = 0.3, MeOH).

Hydrolysis of 2. 10 mg of **2** was dissolved in 20 mL of 0.1 M HCl and refluxed for 90 min. The solvent was removed in a rotary evaporator, 20 mL of water was added, and the solvent was evaporated again. Another 10 mL of water was added, and the mixture was extracted 3 times with 2 mL of *tert*-butyl methyl ether. The *tert*-butyl methyl ether fractions were combined and evaporated in a rotary evaporator, and the solid material obtained (3 mg) was tested on HPLC using the same conditions as for the isolated compound **1**. According to the retention time, and the UV spectra recorded with the DAD, the aglycon part of **2** is 5,8-dihydroxybenzopyranone. The aqueous solution was freeze-dried and a specific optical rotation $[\alpha]_D^{20}$ in water of the remaining material of +20° was obtained.

RESULTS AND DISCUSSION

DPPH Radical Scavenging. The yields of various fractions and their DPPH radical scavenging data, calculated by formula (1), are presented in **Table 1**. The results show that the acetone oleoresin was a more effective radical scavenger than the methanol−water extract. This finding suggests that the most active radical scavengers in sweet grass are rather nonpolar compounds.

Similar results were obtained during the screening of the different fractions. The highest radical scavenging activity was shown by the fraction obtained with *tert*-butyl methyl ether. The activity of this fraction was comparable with that of rosmarinic acid (scavenging = 90%). The activity of the other fractions derived from the methanol−water extract was considerably lower. Although the fractions derived from the acetone oleoresin were more active than those of the methanol−water extract, this was only because of lower amounts of polar compounds in acetone fractions. Because of this the yields of acetone fractions were considerably lower.

Separation of Active Compounds. On the basis of the DPPH screening results, the *tert*-butyl methyl ether fraction of methanol−water−acetic acid extract was selected for further fractionation, separation, and identification of radical scavengers. The HPLC separation of the *tert*-butyl methyl ether fraction was performed with on-line detection with DPPH solution. It can be observed (**Figure 1**) that there is only one compound possessing free radical scavenging activity. As this compound **1** was quite nonpolar, it was purified on silica gel, yielding 141 mg of pure compound. The structure of the compound was determined by NMR and mass spectral data.

The ^{1}H NMR spectrum of **1** (**Table 2**) showed four doublets in the low field ppm range. This indicated that the compound

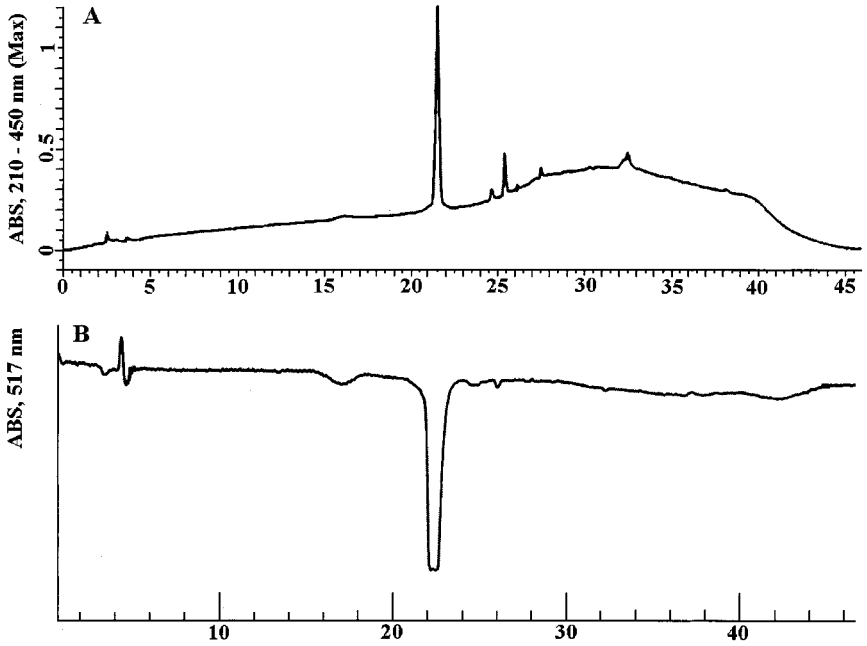

Figure 1. HPLC UV (A) and DPPH (B) on-line chromatograms of the *tert*-butyl methyl ether fraction.

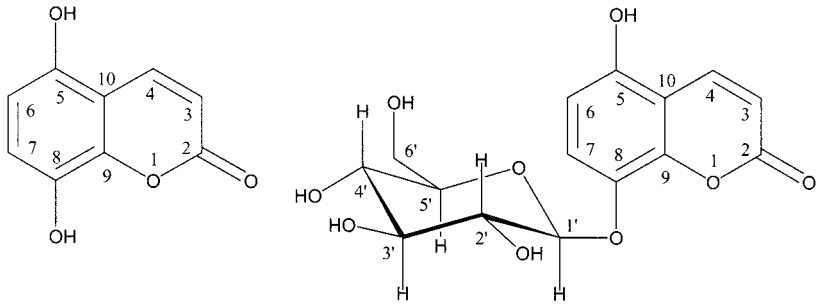

Figure 2. Radical scavengers isolated from *Hierochloe odorata*.

had four nonexchangeable hydrogen atoms in aromatic rings or in a conjugated system. The ^{13}C NMR spectrum showed nine carbon atoms. In combination with the HRMS data (M^+, 178.0273) a molecular formula of $C_9H_6O_4$ (calculated M^+ 178.0266) was determined for this compound. A peak of a carboxylic carbon (162.3 ppm) was present in the ^{13}C NMR spectrum.

From the data obtained it was concluded that the compound possessed a coumarin structure (benzopyranone) with two hydroxy groups attached. The coupling constants of the H atoms suggested that there are two pairs of vicinal protons. As could be seen from the ^{1}H NMR spectrum (two pairs of doublets), there could not be any hydroxy groups in the heteroaromatic ring. Thus, both hydroxy groups were in the benzene ring and only three possible structures for **1** remained. The hydroxyls could be attached to carbons 5 and 6, 5 and 8, or 7 and 8 (**Figure 2**). On the basis of chemical shift evidence in the ^{13}C NMR spectrum, the known 7,8-dihydroxy isomer (daphnetin) could be excluded (*18, 19*). To determine the exact position of the hydroxyl groups, a long-range two-dimensional C−H NMR (COLOC) spectrum was recorded. From the cross-peaks seen in the two-dimensional NMR (**Figure 3**) both the 5,6- and 5,8-substitution patterns were in accordance with the data obtained from this experiment.

To distinguish between the two remaining possibilities a deuteration experiment was performed (*20*). The crux of this experiment is that carbons near a hydroxyl group appear as two peaks due to an isotope effect. Replacement of a hydrogen by a deuterium atom causes a 0.15 ppm shift at the ipso carbon and a 0.05 ppm shift at the neighboring carbons. Six carbon peaks were split because of an exchange of H and D at the hydroxyls (**Figure 4**). This establishes the structure with the hydroxyls at the 5,8 position, i.e., 5,8-dihydroxycoumarin **1**.

In the literature 5,8-dihydroxycoumarin has once before been reported, as a compound obtained from its dimethoxy derivative after dealkylation (*21*). However, the ^{13}C NMR data of the supposed 5,8-dimethoxycoumarin are not in agreement with solid, earlier-reported ^{13}C NMR data on this compound (*19*). Comparing data provided in the above-mentioned reports, it can be concluded that the initial compound, used by Dopke et al. (*21*), to obtain dihydroxycoumarin was in fact not 5,8-dimethoxycoumarin, but its 7,8-dimethoxy isomer. Thus, to our knowledge, this is the first time that this compound is reported as a natural product. The *para*-phenolic groups can readily explain the radical scavenging activity of **1**.

No other active fractions were found in the *tert*-butyl methyl ether fraction, therefore the next experimental step was fractionation of the butanol fraction of sweet grass methanol−water−acetic acid extract.

After separation, 62 mg of pure **2** was obtained, and various spectroscopic data were recorded. The ^{1}H NMR spectrum

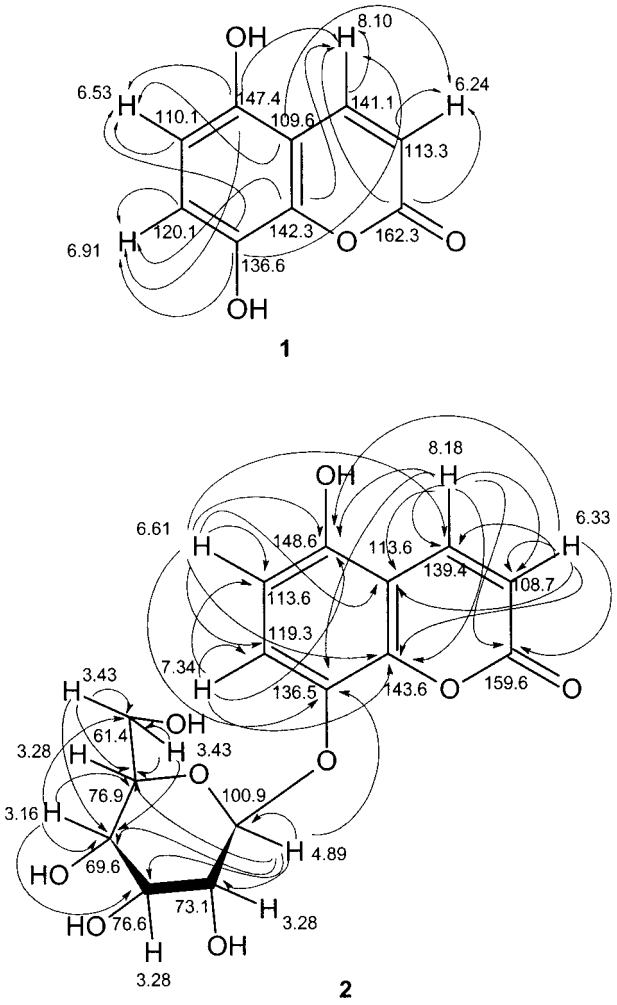

Figure 3. 2D NMR (COLOC) interactions of 5,8-dihydroxycoumarin (**1**) and HMBC interactions of 5-hydroxy-8-*O*-β-D-glucopyranosyl benzopyranone (**2**).

(**Table 2**) of **2** was quite similar to that of 5,8-dihydroxycoumarin, except that there was a doublet at 4.89 ppm, two doublets at 3.43 and 3.46 ppm, a 3H multiplet at 3.3 ppm, and a triplet at 3.16 ppm, suggesting a hexose residue. On the basis of HRMS results (M^+ 340.0796) a molecular formula of $C_{15}H_{16}O_9$ (calculated M^+ 340.0794) was proposed for **2**. The *m*/*z* value of 340 corresponds to the molecular mass of a dihydroxycoumarin with a hexose attached. HRMS confirmed the elemental composition of these two fragments. The accurate mass corresponding to the aglycon part was recorded at 178.0263 ($C_9H_6O_4$, calculated mass 178.0266). On the basis of the ^{1}H and ^{13}C NMR chemical shifts and the 7.7 Hz coupling between H1′ and H2′ (**Table 2**) the hexose was identified as β-glucopyranose. To

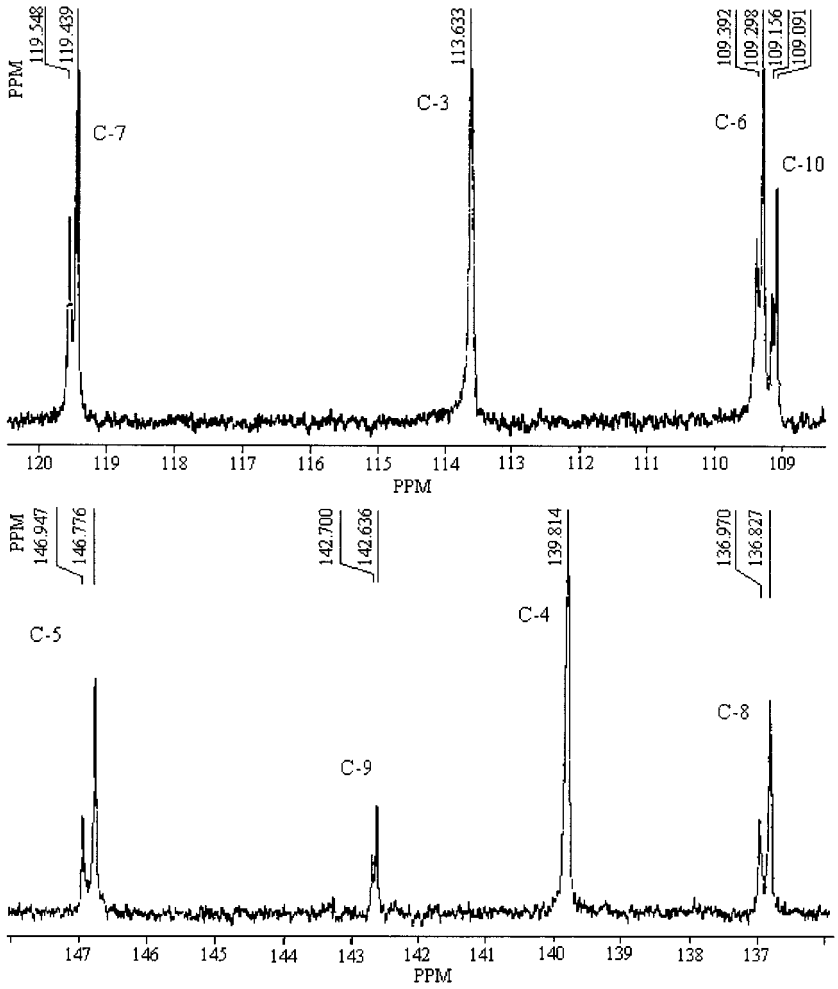

Figure 4. ^{13}C NMR results of deuteration experiment in $CDCl_3$–CD_3OD–CH_3OH.

Table 3. ABTS and DPPH Radical Scavenging Activity of Isolated Compounds in Comparison to the Known Natural Antioxidant Rosmarinic Acid

compound	$TEAC_{1min}$	$TEAC_{6min}$	ARP_{30min}
5,8-dihydroxybenzopyranone	0.62	0.73	8.2
5-hydroxy-8-*O*-β-D-glucopyranosyl-benzopyranone	0.58	0.69	0.8
rosmarinic acid	1.49	1.54	6.9

determine whether the glucose unit was attached to the C5 or C8 hydroxyl group, an HMBC spectrum was recorded. HMBC correlations of compound **2** are presented in **Figure 3**. Some interactions in the glucose moiety are not depicted because of the overlapping of several glucose protons in the ^1H NMR. A cross-peak between C8 of the aglycon and H1′ of glucose was present. Hydrolysis of **2** gave as products **1** and β-D-glucopyranose providing further proof about compound **2**. Thus, the glycoside was identified as 5-hydroxy-8-*O*-β-D-glucopyranosyl-benzopyranone (**Figure 2**). The one remaining phenolic group is presumably responsible for the radical scavenging activity of **2**.

Radical Scavenging Activities of Isolated Compounds. Both isolated compounds were tested for ABTS and DPPH radical scavenging activity (**Table 3**). The ABTS method gives the radical scavenging activity by measuring the reduction of the radical cation as the percentage decrease of absorbance at 734 nm relative to that of a control. In this case the values were observed after 1 and 6 min from the start of the experiment. The activities of the tested compounds were compared with the activity of Trolox and expressed as TEAC values (*15*). In the other method the percentage of DPPH scavenging as a function of the concentration of a test substance was determined. The concentration of compound in the reaction mixture needed to decrease the initial DPPH concentration by 50% was calculated and expressed as antiradical power. It was assumed that the reaction reaches the steady state after 30 min.

The results show that tested compounds act differently in the two systems. In the ABTS system they possess similar activity and both are much less active (TEAC $\approx$ 0.7) than rosmarinic acid ($TEAC_{6min}$ = 1.54). In contrast, in the DPPH system the aglycon was 10 times more powerful as an antioxidant than the glycoside, and its antiradical power (8.2) is comparable to that of rosmarinic acid (6.9) (*14*). This finding proves that the ability of the compound to act as an antioxidant is dependent on the system used. Further experiments in real food systems will be carried out to obtain more information on their antioxidant properties. Because simple coumarins are reported to be of low toxicity (*22*), an application of sweet grass extracts or isolated coumarins as antioxidants in food may be possible.

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ABBREVIATIONS USED

DPPH, 2,2-diphenyl-1-picrylhydrazyl hydrate; BHT, 2,6-*di-tert*-butyl hydroxy toluene; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diamonium salt; PBS, phosphate-buffered saline; TEAC, Trolox equivalent antioxidant coefficient.

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