Antioxidative Phenolic Glycosides from Sage (*Salvia officinalis*)

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An investigation of S*alvia officinalis* L. has led to the isolation of three new phenolic glycosides, 6-*O*-caffeoyl-*β*-D-fructofuranosyl-(2→1)-α-D-glucopyranoside (**1**), 1-*O*-caffeoyl-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**2**), and 1-*O*-*p*-hydroxybenzoyl-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**3**). Elucidation of the structures of **1**−**3** was based on the interpretation of FABMS and 1D and 2D NMR spectra. Compounds **1** and **2** were found to be moderately active as antioxidants in the DPPH test and metmyoglobin test.

Introduction

Salvia officinalis L., a Lamiaceae family spice commonly known as sage (Dalmatian sage), is used in foods for flavoring and seasoning. Its extracts are also well-known for their antioxidative activity.[1] The main antioxidative effect of it was reported to relate to the presence of carnosic acid, carnosol, and rosmarinic acid.[2] In addition, other diterpenes, triterpenes and flavonoids, have been isolated from sage.[3−7]

As part of our studies on cancer chemopreventive compounds from spices, we recently reexamined the chemical and antioxidative components of sage. This paper deals with the isolation and structural elucidation of three new phenolic glycosides from sage and their antioxidant activity.

Results and Discussion

The *n*-butanol fraction of sage extracts was fractionated by a combination of Si gel column chromatography and gel filtration on Sephadex LH-20 and RP-18 columns to yield three new compounds, their structures were elucidated by interpretation of 1D and 2D NMR spectra and comparison with literature data.

Compound **1** (Figure 1) was isolated as colorless crystals. Its molecular formula $C_{21}H_{28}O_{14}$ was deduced from FABMS and ^{13}C NMR. The IR spectrum showed absorption bands due to hydroxyl (3363 cm^{-1}) and carbonyl groups (1688 cm^{-1}). The ^1H NMR spectrum contained the signals for three aromatic protons at δ 7.06 (1H, s), 7.03 (1H, d, J = 8.0 Hz), and 6.76 (1H, d, J = 8.0 Hz), corresponding to a typical 1,2,4-trisubstituted phenyl group. In addition, the ^1H NMR also showed the signals for two trans double bond protons at δ 7.48 (1H, d, J = 16.4 Hz) and 6.31 (1H, d, J = 16.4 Hz), suggesting a *trans*-caffeoyl moiety[8] in compound **1**. This was supported by the ^{13}C NMR spectrum, which in the low field showed the signals for a caffeoyl moiety at δ 167.0 (s), 148.7 (s), 146.8 (d), 146.5 (s), 125.7 (s), 121.7 (d), 116.0 (d), 115.3 (d), 114.2 (d). The ^{13}C NMR also showed the other 12 carbon signals, arising from a disaccharide, the two anomeric carbon signals at δ 104.2 and 91.7, and three CH_2 groups at δ 64.2, 63.0, and 62.5, which suggested this disaccharide was sucrose.[9] This was proved by ^1H NMR spectrum, in view of the characteristic doublet signal with a small coupling constant at δ 5.21 (1H, J = 3.6 Hz), assignable to the anomeric proton in the α-D-glucopyranose unit.

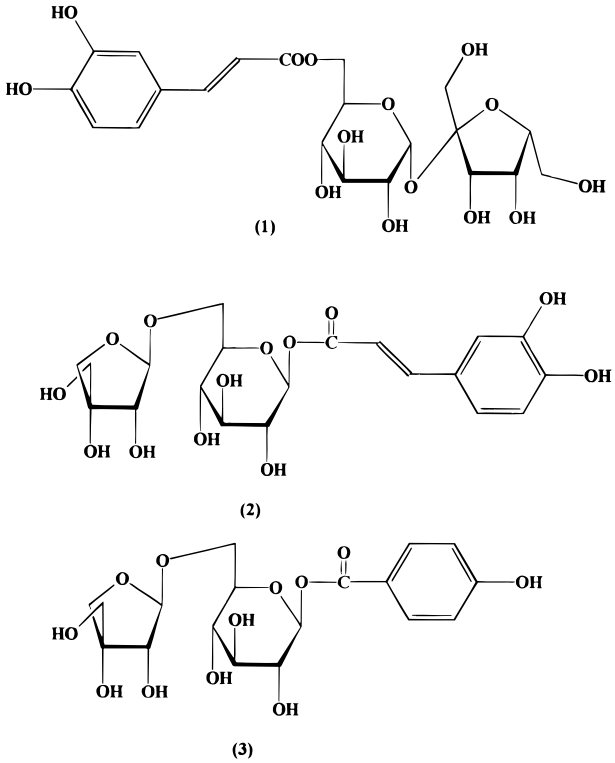

Figure 1. Structure of 6-*O*-caffeoyl-*β*-D-fructofuranosyl-(2→1)-α-D-glucopyranoside (**1**), 1-*O*-caffeoyl-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**2**), and 1-*O*-*p*-hydroxybenzoyl-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**3**).

The linkage of caffeoyl moiety with sucrose moiety was solved by analysis of the HMBC spectrum. In the HMBC spectrum, the carbonyl group (δ 167.0) not only showed correlation with protons assigned to double bond but also had correlation with 6′-methylene protons at δ 4.12 (dd, J = 12.0, 6.4 Hz) and 4.34 (1H, d, J = 12.0 Hz), suggesting the caffeoyl moiety is connected to the 6′-methylene carbon. Compound **1** was therefore elucidated as 6-*O*-caffeoyl-*β*-D-fructofuranosyl-(2→1)-α-D-glucopyranoside. The assignment of protons and carbons of **1** were based on the results

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Table 1. ^{13}C NMR Data of Compounds **1**−**3** (δ in ppm)

C no.	**1**[a,b]	**2**[a,c]	**3**[a,c]
1	125.7 (s)	127.9 (s)	121.8 (s)
2	115.3 (d)	115.6 (d)	133.6 (d)
3	146.5 (s)	147.1 (s)	116.5 (d)
4	148.7 (s)	150.2 (s)	164.3 (s)
5	116.0 (d)	116.8 (d)	116.5 (d)
6	121.7 (d)	123.6 (d)	133.6 (d)
7	146.8 (d)	148.6 (d)	167.0 (s)
8	114.2 (d)	114.7 (d)	
9	167.0 (s)	167.9 (s)	
1′	91.7 (d)	96.0 (d)	96.2 (d)
2′	71.8 (d)	74.2 (d)	74.3 (d)
3′	73.0 (d)	78.2 (d)	78.3 (d)
4′	70.6 (d)	71.6 (d)	71.6 (d)
5′	70.3 (d)	78.0 (d)	78.0 (d)
6′	64.2 (t)	68.7 (t)	68.7 (t)
1″	62.5 (t)	111.3 (d)	111.3 (d)
2″	104.2 (s)	78.2 (d)	78.2 (d)
3″	77.2 (d)	80.9 (s)	80.9 (s)
4″	74.8 (d)	75.3 (t)	75.3 (t)
5″	83.0 (d)	65.9 (t)	65.9 (t)
6″	63.0 (t)		

[a] Measured at 50 MHz. [b] Measured in DMSO-d_6. [c] Measured in CD_3OD.

of the ^{1}H−^{1}H COSY, HMQC, HMBC, ROESY, and TOCSY experiments. The chemical shifts of carbons are listed in Table 1.

Compound **2** was isolated as colorless crystals and exhibited a prominent quasimolecular ion peak in the positive ion FAB mass spectrum at *m/z* 497 $[M + Na]^+$, indicating a molecular mass of 474. Its IR spectrum showed a hydroxyl group (3402 cm^{-1}), a conjugated ester group (1709 cm^{-1}), and an aromatic ring (1612; 1522 cm^{-1}). Like compound **1**, the ^{1}H NMR of compound **2** also showed the signals for a caffeoyl moiety at δ 7.64 (1H, d, J = 16.0 Hz), 7.05 (1H, d, J = 1.8 Hz), 6.97 (1H, dd, J = 8.2, 1.8 Hz), 6.76 (1H, d, J = 8.2 Hz), 6.29 (1H, d, J = 16.0 Hz), but the signals for sugar moiety were different; among them, one signal at δ 4.96 (1H, d, J = 2.4 Hz) is assignable to the anomeric proton of apiose while the signal at δ 3.54 (2H, s) is assignable to 5-position proton of apiose. The ^{13}C NMR spectrum showed signals at δ 111.3 (d), 80.9 (s), 78.2 (d), 75.3 (t), and 65.9 (t), suggesting a terminal β-apiofuranose moiety.[10] Inspection of the remaining carbon signals suggested another sugar was β-glucopyranose. Then by comparison with the reported data,[10,11] the sugar portion was determined to be β-D-apiofuranosyl-(1→6)-β-D-glucopyranose. This was further supported by HMBC experimentation, which showed that a 6-positon carbon of the glucose moiety correlates with an anomeric proton (δ 4.96) of the apiose moiety. In addition, the linkage of the caffeoyl moiety with the sugar portion was also inferred from the HMBC spectrum, in which the ester group at δ 167.9 correlated with a proton at δ 5.53 (1H, d, J = 7.4 Hz) assigned to the H-1′ position of sugar moiety. Compound **2** was identified as 1-*O*-caffeoyl-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside. The total assignment of its protons and carbons was deduced from its ^{1}H−^{1}H COSY, HMQC, HMBC, ROESY, and TOCSY spectra. The chemical shifts of carbons are listed in Table 1.

Compound **3** was isolated as a colorless oil. Its molecular formula $C_{18}H_{24}O_{12}$ was obtained from FABMS and ^{13}C NMR data. Comparison of the ^{1}H NMR and ^{13}C NMR data with those of compound **2** suggested the same sugar portion as in compound **3**, but the signals for the caffeoyl moiety were not observed. Instead signals for a *p*-hydroxybenzoyl group[8] were found. In the ^{1}H NMR spectrum, the signals were at δ 7.97 (2H, d, J = 8.6 Hz) and 6.85 (2H, d, J = 8.6 Hz), while in the ^{13}C NMR spectrum, the signals were at δ 167.0 (s), 164.3 (s), 133.6 (d), 121.8 (s), and 116.5 (d). Therefore, compound **3** was determined to be 1-*O*-*p*-hydroxybenzoyl-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside. This structure was further confirmed by HMBC, HMQC, ^{1}H−^{1}H COSY, ROESY, and TOCSY experiments. The chemical shifts of carbons are assigned as listed in Table 1.

The antioxidant activity of compounds **1**−**3** was studied in two test models. In the DPPH free-radical scavenging test, compounds **1** and **2** showed moderate activity with IC_{50} 20.1 and 19.8 μM, respectively.

The second method we used to test antioxidant activity was the metmyoglobin assay. This method measures the relative ability of antioxidant substances to scavenge the radical cation of 2,2′-azinobis(3-ethylbenzothiozoline-6-sulfonate) ($ABTS^{\bullet+}$) in the aqueous phase as compared to a standard amount of the synthetic antioxidant Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), the water-soluble vitamin E analogue.[12,13] In this assay, the activity of tested compounds was expressed as Trolox equivalent—the millimolar concentration of a Trolox solution having an antioxidant capacity equivalent to 1.0 mM solution of the substance under investigation. In this test, all these compounds have antioxidative activity as compared with Trolox and with Trolox equivalent, 3.70, 3.86, and 0.78, respectively.

Experimental Section

General Experimental Procedures. Melting points were determined on a Thomas-Hoover "unimelt" apparatus and were uncorrected. IR spectra were recorded with an Perkin-Elmer 1600 FT-IR. ^{1}H NMR and ^{13}C NMR spectra were obtained on a VXR-200 instrument. ^{1}H−^{1}H COSY, NOESY, ROESY, TOCSY, HMQC, and HMBC were performed on a Bruker ARX-400 instrument. FAB mass spectra were recorded on a Finnigan MAT-90 instrument. Thin-layer chromatography was performed on Sigma−Aldrich TLC plates (250 μm thickness, 2−25 μm particle size), with compounds visualized by spraying with 5% (v/v) H_2SO_4 in ethanol solution.

Plant Material. Sage was a gift from Kalsec, Inc. (Kalamazoo, MI) and was collected from South Carolina in 1996. A voucher specimen was deposited in the Department of Food Science, Cook College, Rutgers University.

Extraction and Isolation. The dried leaves of sage (30 kg) were extracted with 95% ethanol for 2 weeks. The extract was concentrated to dryness under reduced pressure, the residue (1.5 kg) was dissolved and suspended in water (2.5 L) and partitioned with hexane (3 × 3 L), and then the water layer was extracted with ethyl acetate (3 × 3 L) and *n*-butanol (3 × 3 L). The *n*-butanol extract was evaporated in vacuo to give a residue of 320 g. The residue was subjected to column chromatography (CC) on silica gel (2.0 kg), eluted with $CHCl_3$−MeOH as eluent with increasing MeOH content (20:1, 15:1, 10:1, 9:1, 7:1, 5:1, 4:1, 2:1, 1:1, each 5000 mL), and 1000 mL fractions were collected. A total of 45 fractions were collected.

Fraction 35 (5 g) was subjected to a Lichroprep RP-18 column (100 g) eluted with methanol−water (1:3 1000 mL, 3:2 600 mL) and methanol (500 mL) to get three fractions. Fraction I (400 mg) was rechromatographed on a silica gel column (40 g) and eluted with $CHCl_3$−CH_3OH−H_2O (5:1:0.1) to get 200 mg of compound **1**. Fraction II (720 mg) was first subjected to a Sephadex LH-20 (50 g) column (eluted with methanol) to get two fractions (1 and 2); fraction 2 was then purified with silica gel column (40 g) eluted with $CHCl_3$−CH_3OH−H_2O, (5:1:0.1) to get three subfractions. Subfraction 3 (200 mg) was purified on a silica gel column (40 g) with EtOAc−CH_3OH−H_2O (7:1:1) to get 35 mg of compound **3** and 140 mg of compound **2**.

6-*O*-Caffeoyl-β-D-fructofuranosyl-(2→1)-α-glucopyranoside: colorless crystals (methanol); mp 210−212 °C; $[\alpha]^{23}_D$

+27.17 °C (*c* 0.13, MeOH); IR (film) ν_{max} 3363, 2927, 1688, 1643, 1616, 1532, 1371, 1066, 992 cm^{-1}; FABMS (positive-ion model) *m/z* 527 $[M + Na]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 7.48 (1H, d, J = 16.4 Hz, H-7), 7.06 (1H, s, H-2), 7.03 (1H, d, J = 8.0 Hz, H-6), 6.76 (1H, d, J = 8.0 Hz, H-5), 6.31 (1H, d, J = 16.4 Hz, H-8), 5.21 (1H, d, J = 3.6 Hz, H-1′), 4.34 (1H, d, J = 12.0 Hz, H-6′), 4.12 (1H, dd, J = 12.0, 6.4 Hz, H-6′), 3.99 (1H, t, J = 8.8 Hz, H-5′), 3.89 (1H, t, J = 7.6 Hz, H-3′′), 3.79 (1H, m, H-4′′), 3.62 (1H, m, H-5′′), 3.60 (2H, m, H-6′′), 3.50 (1H, m. H-3′), 3.40 (2H, m, H-1′′), 3.25 (1H, dd, J = 8.8, 4.4 Hz, H-2′), 3.12 (1H, m, H-4′).

1-*O*-Caffeoyl-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside: colorless crystals (methanol); mp 69−71 °C; $[\alpha]^{23}_D$ −21.85 °C (*c* 0.15, MeOH); IR (film) ν_{max} 3402, 2933, 1709, 1612, 1522, 1269, 1066 cm^{-1}; FABMS (positive-ion model) *m/z* 497 $[M + Na]^+$; ^1H NMR (200 MHz CD_3OD): δ 7.64 (1H, d, J = 16.0 Hz, H-7), 7.05 (1H, d, J = 1.8 Hz, H-2), 6.97 (1H, dd, J = 8.2, 1.8 Hz, H-6), 6.76 (1H, d, J = 8.2 Hz, H-5), 6.29 (1H, d, J = 16.0 Hz, H-8), 5.53 (1H, d, J = 7.4 Hz, H-1′), 4.96 (1H, d, J = 2.4 Hz, H-1′′), 3.94 (1H, d, J = 12.0 Hz, H-6′), 3.92 (1H, d, J = 10.0 Hz, H-4′′), 3.89 (1H, d, J = 2.4 Hz, H-2′′), 3.73 (1H, d, J = 10 Hz, H-4′′), 3.62 (1H, m. H-6′), 3.54 (2H, s, H-5′′), 3.47 (1H, m, H-3′), 3.44 (1H, m, H-2′), 3.34 (1H, m, H-5′), 3.29 (1H, m, H-4′).

1-*O*-*p*-Hydroxybenzoyl-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside: colorless oil; $[\alpha]^{23}_D$ −32.75 °C (*c* 0.04, MeOH); FABMS (positive-ion model) *m/z* 455 $[M + Na]^+$; ^1H NMR (200 MHz, CD_3OD) δ 7.96 (2H, d, J = 8.6 Hz, H-2 and H-6), 6.85 (2H, d, J = 8.6 Hz, H-3 and H-5), 5.66 (1H, d, J = 7.4 Hz, H-1′), 4.97 (1H, d, J = 2.6 Hz, H-1′′), 3.96 (1H, d, J = 12.0 Hz, H-6′), 3.92 (1H, d, J = 10.0 Hz, H-4′′), 3.90 (1H, d, J = 2.2 Hz, H-2′′), 3.74 (1H, d, J = 10 Hz, H-4′′), 3.60 (1H, m, H-6′), 3.57 (2H, s, H-5′′), 3.47 (1H, m, H-3′), 3.44 (1H, m, H-2′), 3.33 (1H, m, H-5′), 3.30 (1H, m, H-4′).

Determination of the Scavenging Effect on DPPH Radicals. DPPH (radical 2,2-diphenylpicryhydrazyl) were prepared in ethanol as a 1.0×10^{-4} M solution. This DPPH solution was mixed with different concentrations of compound and kept in a dark area for 0.5 h. The absorbance of the samples was measured on a spectrophotometer (Milton Roy, model 301) at 517 nm against a blank of ethanol without DPPH. All tests were run in triplicate and averaged.

Measurement of Trolox Equivalent Antioxidant Activity (the Metmyoglobin Test). The Trolox equivalent antioxidant activity was measured using commercial kits from Randox Laboratories Ltd. (San Francisco, CA). Phosphate-buffered saline (80 mM) was used as a buffer. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (1.6 mM) was prepared in DMSO for use as an antioxidant standard. Sample solutions (1 mM) were prepared in DMSO. The concentrations in this test were 3.1 μM for metmyoglobin, 305 μM for ABTS, and 125 μM for H_2O_2. First samples or Trolox (20 μL) were mixed with chromogen (1 mL of metmyoglobin and ABTS mixture) in a cuvette and a spectrophotometer (Milton Roy, model 301) was used on 600 nm to get initial absorbance A_1. Then, 200 μL of H_2O_2 was added to this cuvette and held at 40 °C for exactly 15 min before the absorbance A_2 was read. The following calculation was used to determine the Trolox equivalent:

$$(\text{mM}) = \text{Trolox concentration (mM)} \times (\Delta A_{blank} - \Delta A_{sample})/(\Delta A_{blank} - \Delta A_{trolox})$$

$$\Delta A = A_2 - A_1$$

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