Isolation and Structural Elucidation of Two New Glycosides from Sage (*Salvia officinalis* L.)

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Six compounds, 1-*O*-(2,3,4-trihydroxy-3-methyl)butyl-6-*O*-feruloyl-*β*-D-glucopyranoside, ethyl *β*-D-glucopyranosyl tuberonate, *p*-hydroxybenzoic acid, (−)-hydroxyjasmonic acid, caffeic acid, and 4-hydroxyacetophenone 4-*O*-[5-*O*-(3,5-dimethoxy-4-hydroxybenzoyl)-*β*-D-apiofrunosyl]-(1→2)-*β*-D-glucopyranoside, were isolated from the *n*-butanol-soluble fraction of sage leaf extracts. Their structures were determined by spectral methods (MS, NMR, and 2D-NMR), and their antioxidant activities were measured. Among them, two new glycosides were elucidated. All of these compounds showed DPPH free radical scavenging activity at the concentration of 30 mM, and caffeic acid was the most active compound.

Keywords: *Sage; Salvia officinalis L.; glycosides*

INTRODUCTION

Salvia officinalis, a spice commonly known as sage (Dalmatian sage), is a small, evergreen perennial plant with short woody stems that branch extensively. The plant is native to the Mediterranean region and is cultivated all over the world. Dried sage leaves are used as a culinary spice for flavoring and seasoning and as a source of sage oil. In Western countries, sage and its oil have been used for the treatment of various diseases. The name *Salvia* came from the Latin word meaning "to save" or "to heal." A few centuries ago, it was nearly always at the top of the list of household remedies for the relief of itching, the lowering of fevers,and the relief of nervous headaches. Extracts and essential oil have been used to treat digestive disorders and to control excessive sweating (Duke, 1985). Sage has been used internally, as a tea, for the treatment of dysmenorrhea, diarrhea, gastritis, and sore throat. The significant activity of sage extract is its strong antioxidant activity (Chang et al., 1977).

The chemical components of sage are very complex. It is reported to contain 1−2.8% essential oil. The volatile components of sage have been studied during the past two decades (Boelens, 1991). The most important volatile constituents of Dalmatian sage are 1,8-cineole, thujone, isothujone, and camphor. These components may be present at levels of 60% and more in the oil of Dalmatian sage. Diterpenes were reported to be the main phenolic components of sage. Royleanon, 7-α-acetoxyroyleanon, and 6,7-dehydroroyleanon were found to exist in the roots of sage (Brieskorn and Buchberger, 1973). Carnosic acid 12-methyl ether-*γ*-lactone, rosmanol 7-ethyl ether, sagequinone methide A, 6,7-dimethoxyrosmanol, safficinolide, and sageone were isolated from the aerial parts of sage, and safficinolide and sageone have antiviral activity (Tada et al., 1994, 1997; Djarmati et al., 1992). Carnosol, carnosic acid, rosmadial, rosmanol, epirosmanol, and methyl carnosate were identified from sage leaves and showed antioxidant activity, with carnosic acid having the greatest activity (Cuvelier et al., 1994, 1996; Brieskorn and Fuchs, 1662). Flavonoids were also isolated from sage. Genkwanin, luteonin, methoxygenkwanin, hispidulin, 5-methoxysalvigenin, luteonin-7-*O*-glucoside, and apigenin-7-*O*-glucoside were isolated from the leaves of sage, and luteolin was reported to be a strong antimutagen against dietary carcinogen, Trp-P-2 (Samejima et al., 1995; Masterova et al., 1989; Brieskorn and Biechele, 1971; Brieskorn and Kapadia, 1979). Triterpenes, such as α-amyrin, *β*-amyrin, and oleanolic acid, have been found in the leaves of sage (Masterova et al., 1989; Brieskorn and Kapadia, 1980). Other compounds such as pristan and viridiflorol have also been identified from sage (Karl et al., 1982).

Recently we re-examined the chemical components of the *n*-butanol-soluble fraction of sage ethanol extracts (Wang et al., 1998a,b, 1999b). Here we report the isolation and structural elucidation of two novel glycosides, together with four known compounds from the extracts of sage leaves. We also report the DPPH free radical scavenging activity for these compounds.

MATERIALS AND METHODS

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

Extraction and Isolation Procedures. The dried leaves of sage (30 kg) were extracted with 95% ethanol (50 L) at room temperature for 2 weeks. The extract was concentrated to dryness under reduced pressure, and the residue was dissolved in water (2.5 L) and partitioned with hexanes (3 × 3 L). Then the water layer was extracted successively with ethyl acetate (3 × 3 L) and *n*-butanol (3 × 3 L). The water-saturated

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n-butanol extract was evaporated in vacuo at 50 °C to give 320 g of residue. The residue was subjected to column chromatography (CC) on silica gel, eluted with chloroform/methanol as eluent with increasing methanol content (20:1, 15:1, 10:1, 9:1, 7:1, 5:1, 4:1, 2:1, and 1:1, each 5 L), and 1 L fractions were collected and concentrated to dryness under reduced pressure. A total of 45 fractions (fractions 1−45) were collected.

Fraction 7 (2 g) was subjected to a silica gel column using methanol/chloroform (1:20) to give two subfractions; subfraction 1 was rechromatographed on a silica gel column and eluted with ethyl acetate/hexane/acetic acid (1:1:0.1) to give 30 mg of compound **3** and 150 mg of compound **4**.

Fractions 10−12 (20 g) were combined and first subjected to a Sephadex LH-20 column (eluted with methanol) and then rechromatographed on a Lichroprep RP-18 column using methanol/water (3:7) to afford four fractions (I−IV). Fraction II was then subjected to a Lichroprep RP-18 column eluted with methanol/water (1:3 then 3:7) to afford two fractions, II-A and II-B. Fraction II-A was purified with a silica gel column eluted with ethyl acetate/methanol/water (12:1:1) to give 100 mg of compound **5**. Fraction II-B was rechromatographed on silica gel column chromatography and eluted with ethyl acetate/methanol/water (12:1:1), chloroform/methanol (8:1), and ethyl acetate/methanol/water (14:1:1), respectively, to afford 45 mg of compound **2**.

Fraction 18 (10 g) was subjected to an RP-18 column using methanol/water (1:3) as eluent to give four fractions, I−IV. Fraction II was rechromatographed on silica gel column chromatography and eluted with chloroform/methanol/water (7:1:0.1) to get 18 mg of compound **6** and 25 mg of compound **1**.

General Procedures. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Varian 300 instrument (Varian Inc., Palo Alto, CA) and a Varian U-500 instrument (Varian Inc.). ^{1}H−^{1}H COSY, NOESY, HMQC, and HMBC were performed on a Varian U-500 instrument (Varian Inc.). Desorption chemical ionization mass spectra were measured on a JEOL SX-102 mass spectrometer, using ammonia as a reactant gas. APCI-MS analysis was performed on a Micromass Platform II system (Micromass Co.) equipped with a Digital DECPc XL560 computer for data analysis. The ion source temperature was set at 150 °C, and the sample cone voltage was 10V. Thin-layer chromatography was performed on Sigma-Aldrich silica gel TLC plates (250 μm thickness, 2−25 μm particle size), with compounds visualized by spraying with 5% (v/v) H_2SO_4 in an ethanol solution. Silica gel (130−270 mesh), Sephadex LH-20 (Sigma Chemical Co., St. Louis, MO), and a Lichroprep RP-18 column were used for column chromatography. All solvents used for chromatographic isolation were of analytical grade and purchased from Fisher Scientific (Springfield, NJ).

Determination of the Scavenging Effect on DPPH Radicals. DPPH radicals were prepared in ethanol as a 1.0×10^{-4} M solution. This DPPH soluton was mixed with different tested compounds (final concentration was 30 μM) 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.

RESULTS AND DISCUSSION

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

Compound **1** was isolated as a white powder. Its molecular formula, $C_{21}H_{30}O_{12}$, was deduced from negative APCI MS, which showed an $[M - 1]^-$ peak at 473, and ^{13}C NMR, which totally demonstrated 21 carbon signals. The ^{1}H NMR spectrum (Table 1) contained the signals for three aromatic protons at δ 7.19 (1H, d, J = 2.0 Hz), 7.07 (1H, dd, J = 8.1, 2.0 Hz), and 6.80 (1H, d, J = 8.1 Hz), corresponding to a typical 1,2,4-trisubstituted phenyl group. In addition, the ^{1}H NMR also showed the signals for two *trans*-double-bond protons at δ 7.63 (1H, d, J = 15.9 Hz) and 6.40 (1H, d, J = 15.9 Hz) and a methoxy group at δ 3.88 (3H, s), suggesting a *trans*-feruloyl moiety in compound **1** (Zhang et al., 1998; Hamburger and Hostettmann, 1985). This was supported by the ^{13}C NMR spectrum (Table 1), which, in the low field, showed the signals for a feruloyl moiety at δ 169.1 (s), 150.7 (s), 149.4 (s), 147.1 (d), 127.7 (s), 124.2 (d), 116.4 (d), 115.2 (d), 111.7 (d), and 56.5 (q, methoxyl group). The ^{13}C NMR spectrum also showed 11 other carbon signals. Except for one anomeric carbon signal at δ 105.0 and one methyl group at δ 19.6, the remaining nine carbon signals are in the range of 60−80, suggesting a possible sugar and a possible polyalcohol moiety in the molecule. In the ^{1}H NMR spectrum, the signal for the anomeric proton was at δ 4.34 (1H, d, J = 7.8 Hz), suggesting a β-type of sugar with 2-ax H in this compound. ^{1}H−^{1}H COSY, HMQC, and HMBC experiments led to the full assignment of the ^{1}H and ^{13}C signals. The ^{13}C NMR data for the glucose were assigned as δ 105.0 (d, C-1′), 77.7 (d, C-3′), 75.5 (d, C-5′), 75.2 (d, C-2′), 71.6 (d, C-4′), and 64.4 (t, C-6′), matching the reported data of 1,6-disubstituted-β-D-glucose (Damtoft and Jensen, 1994; Abdallah et al., 1994). The polyalcohol moiety was elucidated as $CH_3C(OH)(CH_2OH)CH(OH)CH_2O-$ by studying the remaining ^{1}H and ^{13}C signals and by the correlation from HMBC, HMQC, and ^{1}H−^{1}H COSY spectra.

Table 1. ^{1}H and ^{13}C NMR Data for Compound 1

	^{1}H NMR	^{13}C NMR
1	4.18 (1H, dd, J = 10.1, 2.7 Hz)	72.5 (t)
	3.56 (1H, dd, J = 10.1, 8.6 Hz)	
2	3.80 (1H, dd, J = 8.6, 2.7 Hz)	74.7 (d)
3		74.4 (s)
4	3.42 (1H, d, J = 11.2 Hz)	68.5 (t)
	3.51 (1H, d, J = 11.2 Hz)	
5	1.09 (3H, s)	19.6 (q)
1′	4.34 (1H, d, J = 7.8 Hz)	105.0 (d)
2′	3.25 (1H, dd, J = 9.0, 7.8 Hz)	75.2 (d)
3′	3.40 (1H, dd, J = 9.0, 9.0 Hz)	77.7 (d)
4′	3.37 (1H, dd, J = 9.0, 9.0 Hz)	71.6 (d)
5′	3.54 (1H, m)	75.5 (d)
6′	4.50 (1H, dd, J = 12.0, 2.2 Hz)	64.6 (t)
	4.32 (1H, dd, J = 12.0, 5.6 Hz)	
1″		127.7 (s)
2″	7.19 (1H, d, J = 2.0 Hz)	111.7 (d)
3″		149.4 (s)
4″		150.7 (s)
5″	6.80 (1H, d, J = 8.1 Hz)	116.4 (d)
6″	7.07 (1H, dd, J = 8.1, 2.0 Hz)	124.2 (d)
7″	7.63 (1H, d, J = 15.9 Hz)	147.1 (d)
8″	6.40 (1H, d, J = 15.9 Hz)	115.2 (d)
9″		169.1 (s)
OCH_3	3.88 (3H, s)	56.5 (q)

The linkage of the feruloyl moiety glucose and polyalcohol was solved by analysis of the HMBC spectrum. In the HMBC spectrum, the carbonyl group (δ 169.1) not only showed correlation with protons (δ 6.40, 7.63) assigned to double bonds but also had correlation with 6′-methylene protons at δ 4.50 (dd, J = 12.0, 2.2 Hz) and 4.32 (1H, d, J = 12.0, 5.6 Hz), suggesting the feruloyl moiety is connected to 6′-methylene protons. The anomeric proton at δ 4.34 (1H, d, J = 7.8 Hz) has correlated with 1-methylene carbon at δ 72.5, demon-

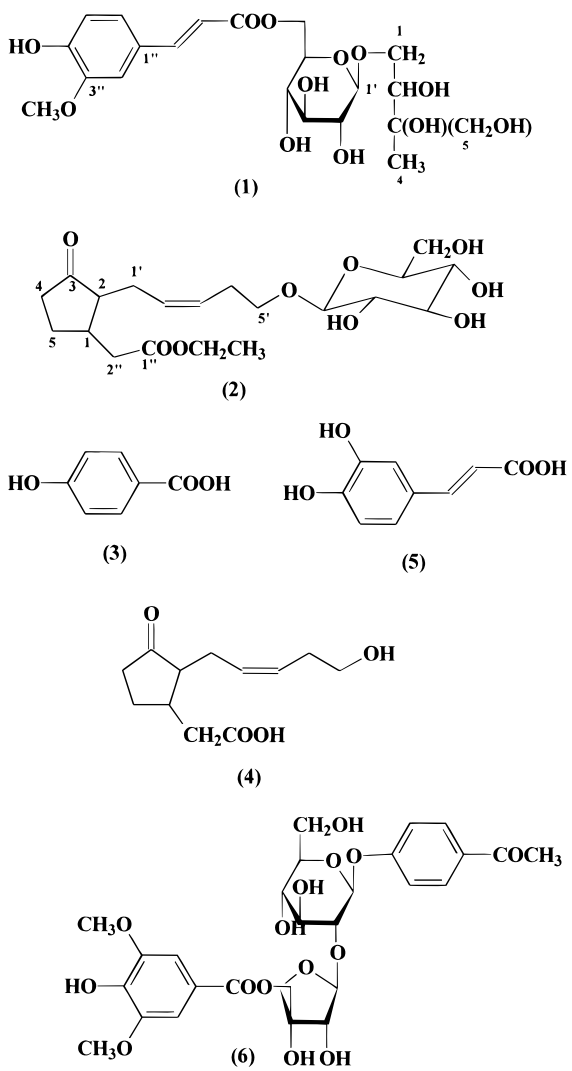

Figure 1. Chemical structures of compounds **1**−**6**.

strating the glucose and polyalcohol have a 1−1′ connection. The structure of this compound, 1-*O*-(2,3,4-trihydroxy-3-methyl)butyl-6-*O*-feruloyl-*β*-D-glucopyranoside is shown in Figure 1.

Compound **2** was isolated as a colorless oil. The molecular formula was established as $C_{20}H_{32}O_9$ by DCI-MS, which showed a molecular ion peak at 434 $[M + NH_4]^+$, and by ^{13}C NMR, which showed a total of 20 signals, comprising two carbonyls, two olefinic methines, and six carbons due to a *β*-D-glucopyranose. ^1H NMR (300 MHz, CD_3OD) showed signals at *δ* 1.27 (3H, t, *J* = 7.0 Hz, $HCOOCH_2\underline{CH_3}$), 1.53 (1H, m, H-5a), 2.00 (1H, dt, *J* = 5.4, 10.7 Hz, H-2), 2.10−2.47 (9H, m, H-1,4,-5b,1′,4′,2″a), 2.72 (1H, dd, *J* = 19.2, 8.4 Hz, H-2″b), 3.18 (1H, dd, *J* = 7.9, 9.2 Hz, H-glc2), 3.33 (3H, H-glc 3,4,5), 3.55 (1H, dt, *J* = 7.0, 9.5, H-5′a), 3.63 (1H, dd, *J* = 11.6, 4.8 Hz, glc-6a), 3.87 (2H, m, H-5′b,6b), 4.15 (2H, q, *J* = 7.0 Hz, $HCOO\underline{CH_2}CH_3$), 4.28 (1H, d, *J* = 7.6 Hz, H-glc1), 5.38−5.50 (2H, m, H-2′,3′). ^{13}C NMR (75 MHz, CD_3OD) showed signals at *δ* 221.1 (s, C-3), 174.6 (s, C-1″), 129.3 (d, C-3′), 129.2 (d, C-2′), 104.6 (d, C-glc1), 78.4 (d, C-glc3), 78.2 (d, C-glc5), 75.4 (d, C-glc2), 71.9 (d, C-glc4), 70.5 (t, C-5′), 63.1 (t, C-glc6), 60.7 ($CO\underline{CH_2}CH_3$), 55.3 (d, C-2), 40.2 (t, C-2″), 39.5 (d, C-1), 38.9 (t, C-4), 29.3 (t, C-4′), 28.4 (t, C-5), 26.7 (t, C-1′). 14.9 (q, $CCOOCH_2\underline{CH_3}$). These NMR data are similar to those for tuberonic acid *β*-D-glucoside (Fujita et al., 1996; Matsuura et al., 1993), except the signals at *δ* 1.27 (3H, t, *J* = 7.0 Hz) and 4.15 (2H, q, *J* = 7.0 Hz) in ^1H NMR and at 14.5 (q) and 60.7 (t) in ^{13}C NMR, which are considered to be from the ethyl group of an ethyl ester moiety. So, compound **2** was elucidated as ethyl *β*-D-glucopyranosyl tuberonate. The antioxidant activity of isolated compounds was measured. At the concentration of 30 *μ*M, all of those compounds showed DPPH radical scavenging activity, with 45.7, 28.8, 32.7, 31.0, 93.2, and 44.2% inhibition for compounds **1**−**6**, respectively. Caffeic acid was the most active compound.

Spectral Identification of Known Compound *p*-Hydroxybezoic Acid (3): white powder; DCI-MS, *m/z* 156 $[M + NH_4]^+$; ^1H NMR (in CD_3COCD_3, 300 MHz) *δ* 7.90 (2H, d, *J* = 8.0 Hz), 6.90 (2H, d, *J* = 8.0 Hz); ^{13}C NMR (CD_3COCD_3, 75 MHz) *δ* 122.6 (s, C-1), 132.7 (d, C-2 and C-6), 115.9 (d, C-3 and C-5), 162.6 (s, C-4), 167.5 (s, C-7).

Spectral Identification of Known Compound (−)-Hydroxyjasmonic Acid (4): DCI-MS, *m/z* 244 $[M + NH_4]^+$; ^1H NMR (in CD_3OD, 300 MHz) *δ* 1.53 (1H, m, H-5a), 2.09−2.37 (10H, m), 2.71 (1H, H-2″a, dd, *J* = 3.6, 14.0 Hz), 3.53 (2H, t, *J* = 7.2 Hz, H-5′), 5,44 (2H, m, H-2′ and H-3′); ^{13}C NMR (CD_3COCD_3, 75 MHz) *δ* 218.6 (s, C-3), 173.8 (s, C-1″), 128.9 (d, C-3′), 128.7 (d, C-2′), 62.1 (t, C-5′), 54.3 (d, C-2), 39.0 (t, C-2″), 38.7 (d, C-1), 38.0 (t, C-4), 31.7 (t, C-4′), 27.5 (t, C-5), 26.1 (t, C-1′). This is identical with the literature (Fujita et al., 1996).

Spectral Identification of Known Compound Caffeic Acid (5): DCI-MS, *m/z* 198 $[M + NH_4]^+$; ^1H NMR (in CD_3OD, 300 MHz) *δ* 7.53 (1H, d, *J* = 16.0 Hz, H-7), 7.04 (1H, d, *J* = 2.1 Hz, H-2), 6.93 (1H, dd, *J* = 8.0, 2,1 Hz, H-6), 6.78 (1H, d, *J* = 8.0 Hz, H-5), 6.22 (1H, d, *J* = 16.0 Hz, H-8); ^{13}C NMR (DMSO-d_6, 75 MHz) *δ* 171.1 (s, C-9), 149.4 (s, C-4), 147.0 (d, C-7), 146.8 (s, C-3), 127.8 (d, C-1), 122.8 (d, C-6), 116.5 (d, C-8), 115.5 (d, C-2), 115.1 (d, C-5). This is identical with the literature (Damtoft and Jensen, 1994).

Spectral Identification of Known Compound 4-Hydroxyacetophenone 4-*O*-[5-*O*-(3,5-Dimethoxy-4-hydroxybenzoyl)-*β*-D-apiofrunosyl]-(1→2)-*β*-D-glucopyranoside (6): white powder; FAB-MS, *m/z* 611 $[M + 1]^+$, 633 $[M + Na]^+$; ^1H NMR (in CD_3OD, 500 MHz) *δ* 7.69 (2H, d, *J* = 9.0 Hz, H-2,6), 6.93 (2H, d, *J* = 9.0 Hz, H-3,5), 2.43 (3H, s, H-8), 5.05 (1H, d, *J* = 7.6 Hz, H-1′), 3.70 (1H, dd, *J* = 9.0, 7.6 Hz, H-2′), 3.63 (1H, dd, *J* = 9.0, 9.0 Hz, H-3′), 3.39 (1H, d, *J* = 9.0 Hz, H-4′), 3.45 (1H, m, H-5′), 3.86 (1H, dd, *J* = 2.2, 12.2 Hz, H-6′a), 3.67 (1H, dd, *J* = 5.6, 12.2 Hz, H-6′b), 5.51 (1H, d, *J* = 0.7 Hz, H-1″), 4.00 (1H, d, *J* = 0.7 Hz, H-2″), 3.90 (1H, d, *J* = 9.5 Hz, H-4″a), 4.27 (1H, d, *J* = 9.5 Hz, H-4″b), 4.18 (1H, d, *J* = 11.2 Hz, H-5″a), 4.33 (1H, d, *J* = 11.2 Hz, H-5″b), 7.14 (2H, s, H-2‴,6‴). This is identical with the literature (Wang et al., 1999a).

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