Isolation and Structural Elucidation of Aroma Constituents Bound as Glycosides from Sage (*Salvia officinalis*)

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Four glycosidic bound flavor precursors, (1*S*,2*R*,4*R*)-1,8-epoxy-*p*-menthan-2-yl-*O*-*β*-D-glucopyranoside, (6*R*,9*R*)-3-oxo-α-ionol *β*-D-glucopyranoside, (6*R*,9*S*)-3-oxo-α-ionol *β*-D-glucopyranoside, and eugenyl-glucoside, were isolated from the ethanol extract of Dalmatian sage. Their structures were elucidated by spectral methods (NMR and MS). All four compounds are new to the constituents of sage.

Keywords: *Sage; flavor precursors; monoterpenes; C_{13} norisoprenoid; Salvia officinalis*

INTRODUCTION

Sage (Dalmatian sage) (*Salvia officinalis*) is a spice commonly used for flavoring and seasoning of foods. The volatile components of sage have been studied during the last two decades (Boelens, 1991). The most important 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.

Many volatile compounds are present in fruits, vegetables, and spices bound to glycosides, from which they can be released during fruit maturation, storage, pretreatment, or processing by enzyme action or acid-catalyzed reaction (Crouzet, 1997). The presence of these compounds was first reported by Bourquelot and Bridel (1913), who identified a geranyl-*β*-D-glucoside in *Pelargonium odoratissimum*. During the last 10 years, the interest in flavor development and release in foods has stimulated a growing research activity in flavor precursors (Williams et al., 1992). Among those precursors, glycosidically bound volatile compounds have been extensively studied (Schwab et al., 1989; Wu et al., 1990; Guldner and Winterhalter, 1991; Krammer et al., 1991; Salles et al., 1991; Adedeji et al., 1992; Marlatt et al., 1992; Humpf and Schreier, 1992).

In the present study, we report the isolation and structural elucidation of four glycosidically bound flavor precursors from nonvolatile fractions of sage extracts.

MATERIALS AND METHODS

General Procedures. ^{1}H NMR and ^{13}C NMR spectra were obtained on a 300 instrument, and ^{13}C NMR multiplicity was determined by DEPT experiments. Desorption chemical ionization mass spectra were measured on a JEOL SX-102 mass spectrometer, using ammonia as reactant gas. 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 an ethanol solution. Silica gel (70−130 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 quality and purchased from Fisher Scientific (Springfield, NJ).

Plant Material. The sage leaves were a gift from Kalsec, Inc. (Kalamazoo, MI).

Extraction and Isolation Procedures. The dried sage leaves (30 kg) were extracted with 95% ethanol for 2 weeks. The extract was concentrated to dryness under reduced pressure, and the residue was then dissolved and suspended in water (2.5 L) and partitioned with hexane (3 × 3 L). 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, 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. Fractions 10−12 (20 g) were combined together and subjected to Sephadex LH-20 column (eluted with methanol) to remove flavanoids and then rechromatographed on a lichroprep RP-18 column using methanol−water (3:7) to get 4 (I−IV) fractions. Fraction II was then subjected to a Lichroprep RP-18 column eluted with methanol−water (1:3 then 3:7) to get two fractions II-A and II-B. Fraction II-A was purified with silica gel column eluted with ethyl acetate−methanol−water (12:1:1) to get 140 mg of compound **1**. Fraction II-B was rechromatographed on silica gel CC, eluted with ethyl acetate−methanol−water (12:1:1), $CHCl_3$−methanol (8:1), and ethyl acetate−methanol−water (14:1:1), respectively, to get 150 mg of compound **2** and 45 mg of compound **3**. Fraction III was first subjected to an RP-18 column using methanol−water (1:3) as eluents and then subjected to silica gel CC eluted with ethyl acetate−methanol−water (20:1:1) to yield 260 mg of compound **4**.

(1*S*,2*R*,4*R*)-1,8-Epoxy-*p*-menthan-2-yl-*O*-*β*-D-glucopyranoside (1). **1** is an amorphous solid, mp 92−94 °C. DCI-MS (*m*/*z*): 350 $[M + NH_4]^+$, 250, 151, 135. ^{1}H NMR (in CD_3OD, 300 MHz): *δ* 1.13 (3H, s, Me-7), 1.26 (3H, s, Me-10), 1.31 (3H, s, Me-11), 1.32−1.57 (3H, m, H-4, H-5 endo, H-6 endo), 1.76 (1H, m H-6 exo), 1.87−2.15 (3H, m, H-5 exo, H-3), 3.17−3.40 (4H, m, H-2′, 3′, 4′, 5′), 3.65 (1H, m, H-6′), 3.95−3.92 (2H, H-6′, m, H-2 endo), 4.38 (1H, d, *J* = 7.8 Hz).

(6*R*,9*S*)-3-Oxo-α-ionol *β*-D-glucopyranoside (2). **2** is an amorphous powder. DCI-MS (*m*/*z*): 388 $[M + NH_4]^+$, 348, 208, 142. ^{1}H NMR (in CD_3OD, 400 MHz): *δ* 0.99 (3H, s, H-12), 1.03 (3H, s, H-11), 1.28 (3H, d, *J* = 6.3, H-10), 1.99 (3H, d, *J* = 0.9, H-13), 2.12 (1H, d, *J* = 15.9 Hz, H-2α), 2.48 (1H, d, *J* = 16, H-2*β*), 2.70 (1H, d, *J* = 9.6, H-6), 3.10−3.50 (4H, m, H-2′, 3′, 4′ 5′), 3.63 (1H, dd, *J* = 11.7, 6.0, H-6′*β*), 3.87 (1H, dd, *J* =

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Table 1. ^{13}C NMR Data (75 MHz, Multiplicity Determined by DEPT ^{13}C NMR; s = Singlet, d = Doublet, t = Triplet, q = Quartet) for Isolated Compounds

posn	compd **1**[a]	compd **2**[a]	compd **3**[b]	compd **4**[a]
1	73.9 s	37.2 s	36.5 s	146.3 s
2	74.9 d	48.2 t	47.9 t	150.7 s
3	31.7 t	202.0 s	198.4 s	114.1 d
4	34.7 d	126.2 d	126.0 d	136.4 s
5	22.7 t	165.6 s	162.3 s	122.1 d
6	31.0 t	56.9 d	55.9 d	118.2 d
7	23.6 q	131.2 d	128.4 d	40.7 t
8	75.7 s	137.0 d	137.7 d	139.0 d
9	29.2 q	74.8 d	74.8 d	115.9 t
10	28.3 q	22.2 q	21.3 q	56.7 q
11		27.4 q	27.5 q	
12		28.0 q	27.9q	
13		23.9 q	23.4 q	
1′	100.5 d	101.2 d	102.2 d	103.0 d
2′	74.9 d	74.9 d	76.5 d	74.9 d
3′	78.1 d	78.4 d[c]	78.0 d	78.1 d[d]
4′	71.9 d	71.7 d	71.5 d	71.3 d
5′	78.1 d	78.2 d[c]	77.2 d	77.8 d[d]
6′	63.0 t	62.6 t	62.8 t	62.5 t

a, measured in CD_3OD. b, Measured in CD_3COCD_3. c,d, assignment may be reversed.

11.7, 2.8, H-6′α), 4.31 (1H, d, J = 7.5, H-1′), 4.48 (1H, m, H-9), 5.59 (1H, dd, J = 15.3, 7.5, H-8), 5,76 (1H, dd, J = 15.6, 9.3, H-7), 5.89 (1H, s, H-4).

(6*R*,9*R*)-3-Oxo-α-ionol *β*-D-glucopyranoside (3). **3** is a colorless oil. DCI-MS (*m*/*z*): 388 $[M + NH_4]^+$, 348, 208, 142. ^{1}H NMR (in CD_3COCD_3, 400 MHz): δ 0.97 (3H, s, H-12), 0.99 (3H, s, H-11), 1.25 (3H, d, J = 6.4, H-10), 1.90 (3H, d, J = 0.9, H-13), 2.05 (1H, d, J = 16.4 Hz, H-2α), 2.36 (1H, d, J = 16.4, H-2*β*), 2.64 (1H, d, J = 9.0, H-6), 3.10−3.50 (4H, m, H-2′, 3′, 4′ 5′), 3.64 (1H, m, H-6′*β*), 3.79 (1H, m, H-6′α), 4.36 (1H, d, J = 7.5, H-1′), 4.43 (1H, m, H-9), 5.63 (1H, dd, J = 15.4, 8.6, H-7), 5.75 (1H, dd, J = 15.6, 6.1, H-8), 5.80 (1H, s, H-4).

Eugenylglucoside (4). **4** is an amorphous powder. DCI-MS (*m*/*z*): 344 $[M + NH_4]^+$, 181, 164, 110. ^{1}H NMR (in CD_3OD, 300 MHz): δ 7.08 (1H, d, J = 8.1, H-6), 6.82 (1H, d, J = 2.1, H-3), 6.72 (1H, dd, J = 8.1, 2.1, H-5), 5.95 (1H, m, H-8), 5.06 (2H, m, H-9), 4.85 (1H, d, J = 7.8, H-1′), 3.87 (1H, m, H-6′), 3.68 (1H, m, H-6′), 3.29−3.52 (4H, m, H-2′, H-3′, H-4′, H-5′).

RESULTS AND DISCUSSION

Four compounds were isolated from the butanol extract of sage, and their structures were elucidated by spectral methods. Compound **1** was isolated as an amorphous powder, and the molecular formula was deduced as $C_{16}H_{28}O_7$ from DCI-MS and ^{13}C NMR. The ^{13}C NMR of compound **1** (Table 1) showed 16 carbon signals, 10 assignable to the oxygenated 1.8-cineole moiety and the remaining 6 carbon signals to a *β*-glucose moiety. The ^{13}C NMR signal of the monoterpene moiety is very similar to those of 2*β*-cinnamoyl cineole and 2*β*-hydroxy-1,8-cineole but different from 2-α-hydroxy-1,8-cineole and 2α-cinnamoyl cineole (Sy and Brown, 1997; Carman and Fletcher, 1983, 1984; Miyazawa et al., 1991), so compound **1** was suggested to be 2*β*-hydroxy-1,8-cineole *β*-D-glucopyranoside (with 2*R* or 2*S* configuration). In a comparison of the ^{1}H NMR and ^{13}C NMR with those of (1*S*,2*S*,4*R*)-1,8-epoxy-*p*-menthan-2-yl-*O*-*β*-D-glucopyranoside, (1*R*,2*R*,4*S*)-1,8-epoxy-*p*-menthan-2-yl-*O*-*β*-D-glucopyranoside (Orihara and Furuya, 1994), and (1*S*,2*R*,4*R*)-1,8-epoxy-*p*-menthan-2-yl-*O*-*β*-D-glucopyranoside (Manns, 1995), compound **1** was elucidated as (1*S*,2*R*,4*R*)-1,8-epoxy-*p*-menthan-2-yl-*O*-*β*-D-glucopyranoside (Figure 1).

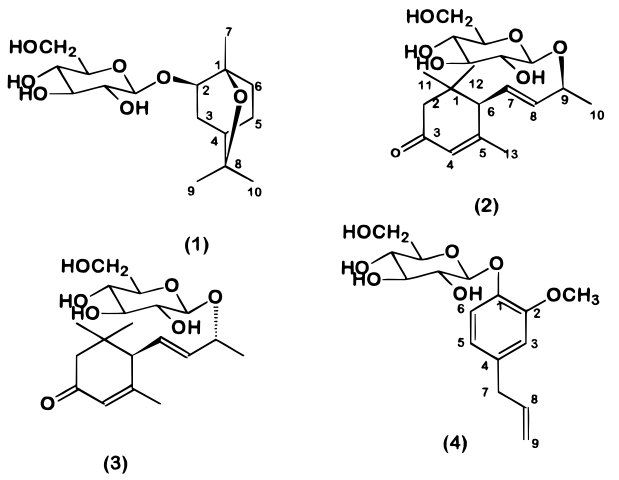

Figure 1. Structures of compounds identified in sage extract: (**1**) (1*S*,2*R*,4*R*)-1,8-epoxy-*p*-menthan-2-yl-*O*-*β*-D-glucopyranoside; (**2**) (6*R*,9*S*)-3-oxo-α-ionol *β*-D-glucopyranoside; (**3**) (6*R*,9*R*)-3-oxo-α-ionol *β*-D-glucopyranoside; (**4**) eugenylglucoside.

Compound **2** was isolated as an amorphous powder, and compound **3** was isolated as a colorless oil. Their DCI-MS showed the same fragments patterns. The presence of peaks at *m*/*z* 388 $[M + NH_4]^+$ and the ^{13}C NMR (Table 1) showing the signals of *β*-glucose suggested that both compounds were monoglycosides. In addition to the signals for glucose, both ^{13}C NMR spectra showed other 13 carbon signals, among them one carbonyl group signal at δ 202.0 for compound **2** and 198.4 ppm for compound **3**. The ^{1}H NMR spectra of both compounds **2** and **3** showed signals for three single methyl groups. This information suggested that both compounds were C-13 norisoprenoids. In comparison of the data with data in the literature (Ho et al. 1990; Pabst et al., 1992), compounds **2** and **3** were elucidated as (6*R*,9*S*)-3-oxo-α-ionol *β*-D-glucopyranoside and (6*R*,9*R*)-3-oxo-α-ionol *β*-D-glucopyranoside (Figure 1), respectively. Their absolute configurations have been established by CD correlation as well as NMR analysis of their corresponding (R)-(−)-α-phenylpropionic acid ester (Pabst et al., 1992).

Eugenylglucoside (**4**) was isolated as an amorphous powder, and the molecular formula was determined as $C_{16}H_{22}O_7$ by DCI-MS and ^{13}C NMR. The ^{13}C NMR (Table 1) showed 16 signals, 6 carbon signals assignable to a *β*-D-glucose, 2 signals (δ 139.0, 115.9 ppm) suggesting one double bond (−CH=CH_2), and 2 resonances at 150.7 and 146.3 ppm suggesting hydroxylated aromatic carbons. The ^{1}H NMR of compound **4** showed signals at 7.08 (1H, d, J = 8.1), 6.82 (1H, d, J = 2.1) and 6.72 ppm (1H, dd, J = 8.1, 2.1) suggesting one 1,3,4-trisubstituted benzene ring. Compound **4** was, therefore, established as eugenylglucoside (Figure 1). All NMR data were identical to those of found in the literature (Mulkens and Kapetanidis, 1988).

Both monoterpene alcohols and C_{13} norisoprenoids are important aroma constituents in many essential oils. Recent studies suggest that many of those compounds are present in plants bound to glycosides. The present study indicates that glycosidic flavor precursors may also play an important role in the flavor of sage. All four glucosides identified in this study are new to the constituents of sage.

LITERATURE CITED

Adedeji, J.; Hartman, T. G.; Lech, J.; Ho, C.-T. Characterization of glycosidically bound aroma compounds in the African mango (*Mangifera indica* L.) *J. Agric. Food Chem*. **1992**, *40*, 659−661.

Boelens, M. H. Spices and condiments II. In *Volatile Compounds in Foods and Beverages*; Maarse, H., Ed.; Dekker: New York, 1991; pp 449−482.

Bourquelot, E.; Bridel, M. Synthese du geranylglucoside β al'aide de l'emulsine sa presence daus les vegetaux. *C. R. Acad. Sci.* **1913**, *157*, 72−74.

Carman, R. M.; Fletcher, M. T. Halogenated terpenoids. XX. The seven monochlorocineoles. *Aust. J. Chem*. **1983**, *36*, 1483−93.

Carman, R. M.; Fletcher, M. T. The isomeric 1,3,3-trimethyl-2-oxabicyclo[2,2,2]octan-6-ols (2-hydroxy-1,8-cineoles). *Aust. J. Chem*. **1984**, *37*, 1117−1122.

Crouzet, J. Flavor biogeneration. In *Functionality of Food Phytochemicals*; Johns, T., Romeo, J. T., Eds.; Plenum Press: New York, 1997; pp 179−200.

Guldner, A.; Winterhalter, P. Structures of two new ionone glycosides from quince (*Cydonia oblonga* Mill.). *J. Agric. Food Chem*. **1991**, *39*, 2142−2146.

Ho, C.-T.; Sheen, L.-Y.; Kuo, M.-C. Glycosidically bound aroma compounds in pineapple. In *Flavour Science and Technology*; Bessière, Y., Thomas, A. F., Eds.; John Wiley & Son: Chichester, U.K., 1990; pp 77−80.

Humpf, H.-U.; Schreier, P. 3-Hydroxy-5,6-epoxy-β-ionol β-D-glucopyranoside and 3-hydroxy-7,8-dihydro-β-ionol β-D-glucopyranoside: New C_{13} norisoprenoid glucoconjugates from sloe tree (*Prunus spinosa* L.) leaves. *J. Agric. Food Chem*. **1992**, *40*, 1898−1901.

Krammer, G.; Winterhalter, P.; Schwab, M.; Schreier, P. Glycosidically bound aroma compounds in the fruits of *Prunus* species: Apricot (*P. armeniaca*, L.), peach (*P. persica*, L.), yellow plum (*P. domestica*, L. ssp. Syriaca). *J. Agric. Food Chem*. **1991**, *39*, 778−781.

Manns, D. Linalool and cineole type glucosides from *Cunila spicata*. *Phytochemistry* **1995**, *39*, 1115−1118.

Marlatt, C.; Ho, C-.T.; Chien, M. Studies of aroma constituents bound as glycosides in tomato. *J. Agric. Food Chem*. **1992**, *40*, 249−252.

Miyazawa, M.; Nakaoka, H., Hyakumachi, M.; Kameoka, H. Biotransformation of 1,8-cineole to (+)-2-endo-hydroxy-1,8-cineole by Glomerella cingulata. *Chem. Express* **1991**, *6*, 667−670.

Mulken, A.; Kapetanidis, I. Eugenylglucoside, a new natural phenylpropanoid heteroside from *Melissa officinalis*. *J. Nat. Prod*. **1988**, *51*, 496−498.

Orihara, Y.; Furuya, T. Biotransformation of 1,8-cineole by cultured cells of *Eucalyptus perriniana*. *Phytochemistry* **1994**, *35*, 641−644.

Pabst, A.; Barron, D.; Semon, E.; Schreier, P. Two diastereomeric 3-oxo-α-ionol β-D-glucosides from raspberry fruit. *Phytochemistry* **1992**, *51*, 1649−1652.

Salles, C.; Jallageas, J.; Fournier, F.; Tabet, J.; Crouzet, J. C. Apricot glycosidically bound volatile components. *J. Agric. Food Chem*. **1991**, *39*, 1979−1983.

Schwab, W.; Mahr, C.; Schreier, P. Studies on the enzymic hydrolysis of bound aroma components from *Carica papaya* Fruit. *J. Agric. Food Chem*. **1989**, *37*, 1009−1012.

Sy, L.-K. Brown, D. G. Oxygenated Bisabolanes from *Alpinia densibracteata*. *Phytochemistry* **1997**, *45*, 537−544.

Williams, P. J.; Sefton, M. A.; Francis, I. L. Glycosidic precursors of varietal grape and wine flavor. In *Flavor Precursors*; Teranishi, T. R., Takeoka, M., Guntert, M., Eds.; ACS Symposium Series 490; American Chemical Society: Washington, DC, 1992; pp 74−86.

Wu, P.; Kuo, M.-C.; Ho, C.-T. Glycosidically bound aroma compounds in ginger (*Zingiber officinale* Roscoe). *J. Agric. Food Chem*. **1990**, *38*, 1553−1555.

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