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Flavonoid and phenolic glycosides from *Salvia officinalis*

Yinrong Lu *, L. Yeap Foo

Industrial Research Limited, PO Box 31310, Lower Hutt, New Zealand

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Abstract

Two novel phenolic glycosides *cis*-*p*-coumaric acid 4-*O*-(2′-*O*-β-D-apiofuranosyl)-β-D-glucopyranoside and *trans*-*p*-coumaric acid 4-*O*-(2′-*O*-β-D-apiofuranosyl)-β-D-glucopyranoside were isolated and identified from *Salvia officinalis* together with 4-hydroxyacetophenone 4-*O*-(6′-*O*-β-D-apiofuranosyl)-β-D-glucopyranoside, luteolin 7-*O*-β-D-glucoside, 7- and 3′-*O*-β-D-glucuronide, 6-hydroxyluteolin 7-*O*-β-D-glucoside and 7-*O*-glucuronide, and 6,8-di-*C*-β-D-glucosylapigenin (vicenin-2). The luteolin glucuronides and vicenin-2 were identified as new sage constituents. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Salvia officinalis*; Labiatae; Phenolic glycosides; Flavonoids; *cis*- and *trans*-*p*-coumaric acid 4-*O*-(2′-*O*-apiosyl)glucoside

1. Introduction

Sage (*Salvia* spp.), a popular herb in the mint family (Labiatae), has been a subject of intensive study in the past decades for its antioxidative components. Sage has been long used in folk medicines for the treatment of all kinds of ailments, but to most people it is better known as an additive used in the preparation of different types of foods. Several studies have shown sage to be one of the sources of some potent antioxidants (Chipault et al., 1952; Cuvelier et al., 1996). The antioxidant properties were found to be related to the presence of rosmarinic acid and carnosic acid (Chang et al., 1977; Cuvelier et al., 1994). More recent studies on sage have revealed the presence of a large number of diterpenoids (Gonzalez et al., 1992; Tang and Eisenbrand, 1992) and phenolic acids (Tanaka et al., 1996, 1997; Li, 1998; Tezuka et al., 1998) including a number of novel caffeic acid metabolites such as sagerinic acid (Lu and Foo, 1999) and sagecoumarin (Lu et al., 1999), but comparably few flavonoids and phenolic glycosides (Ulubelen and Miski, 1981; Gökdil et al., 1997; Wang et al., 1998, 1999). This report deals with the flavonoid and phenolic glycosides from *S. officinalis*.

2. Results and discussion

The HPLC analysis of the 70% aqueous acetone extract of *S. officinalis* showed the presence of a number of phenolic acids and flavonoids, the latter were readily detected by the especially characteristic strong absorption at 340–350 nm. The polar fraction was treated on a column of Sephadex and where necessary the isolated fractions were further purified on a column of MCI HP20 leading to the isolation of three phenolic glycosides (**1–3**) and six flavonoids (**4–9**).

Comparison of the NMR spectra of compound **1** with those published by Wang et al. (1998) identified **1** as 4-hydroxyacetophenone 4-*O*-(6′-*O*-β-D-apiofuranosyl)-β-D-glucopyranoside. The ^{13}C NMR spectrum of **2** also showed 11 carbon signals of an apiose and a glucose moieties as in **1** (see Table 1). However, the downfield position of the glucose C-2 (δ 79.8) suggested the interglycosidic linkage to be 1→2 rather than 1→6 as in **1**. The observation of two doublets at δ 5.4 (J 1.9 Hz) and 5.1 (J 7.1 Hz) in the ^{1}H NMR spectrum of **2** was indicative of the β-configurations for both the apiose and glucose moieties. The aglycone of **2** was identified as a *p*-coumaric acid moiety by NMR comparison with that of *p*-coumaric acid 4-*O*-β-D-glucoside identified before (Foo et al., 2000), suggesting **2** was *trans*-*p*-coumaric acid 4-*O*-(2′-*O*-β-D-apiofuranosyl)-β-D-glucopyranoside. This structural assignment was further confirmed by 2D NMR experiments which showed long range

* Corresponding author. Fax: +64-4-5690055.
E-mail address: y.lu@irl.cri.nz (Y. Lu).

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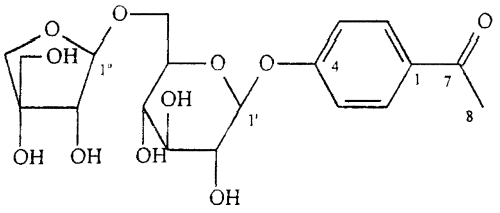

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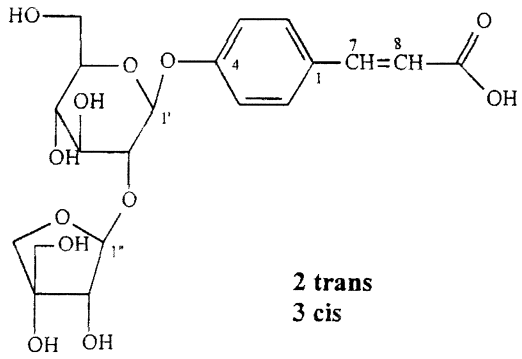

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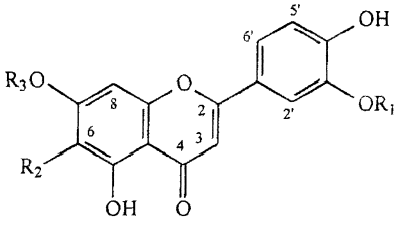

4 R_1=H, R_2=H, R_3=Glu
5 R_1=H, R_2=H, R_3=Glucu
6 R_1=Glucu, R_2=H, R_3=H
7 R_1=H, R_2=OH, R_3=Glu
8 R_1=H, R_2=OH, R_3=Glucu

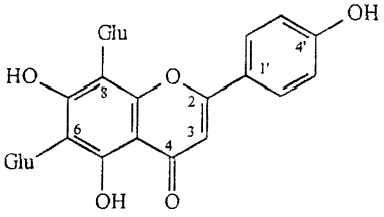

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Table 1
^{13}C NMR data of phenolic glycosides **1**–**3**

C	1	2	3
1	133.08	131.86	131.49
2	132.20	130.87	130.18
3	117.79	118.20	116.46
4	163.41	159.82	156.49
5	117.79	118.20	116.46
6	132.20	130.87	130.18
7	200.39	142.03	130.18
8	27.01	124.23	126.31
9		176.03	177.67
1′	101.94	100.83	99.15
2′	75.17	79.80	79.04
3′	78.43	78.36	76.46
4′	71.92	71.40	69.68
5′	77.55	78.17	76.22
6′	69.21	62.60	60.95
1″	111.37	111.24	109.82
2″	78.26	78.69	77.36
3″	80.92	81.28	79.88
4″	75.37	75.69	74.20
5″	65.86	65.96	64.24

correlations between H-1′ (δ 5.1) and C-4 (δ 159.8) and between H-1″ (δ 5.4) and C-2′ (δ 79.8). Compound **3** was the *cis*-isomer of **2** based on the observations that **2** or **3** were interconvertible on UV radiation. The *cis*-orientation in **3** was apparent from the ^{1}H NMR spectrum which showed two doublets at δ 6.0 and 6.4 (J 12.6 Hz). The two doublets at δ 5.2 (J 7.3 Hz) and 5.4 (J 2.1 Hz) were consistent with the β-configurations of the glucose and apiose concerned and the 1→2 interglycosidic linkage was deduced from the downfield shift of the glucose C-2 to 79.0 ppm. As far as can be ascertained the *cis*- and *trans*-*p*-coumaric acid 4-*O*-(2′-*O*-β-D-apiosyl)-β-D-glucosides have not been described previously as natural compounds and phenolic apiosylglucosides with 1→2 interglycosidic linkages have not been reported in sage, although those with 1→6 interglycosidic linkages were found recently in *S. officinalis* (Wang et al., 1998, 1999).

Compounds **4**–**9** all showed a singlet at around 6.7 ppm in their ^{1}H NMR spectra consistent with the H-3 of flavones and this was supported by the observation of carbon signals at ca 103 ppm associated with the C-3 in their ^{13}C NMR spectra (see Table 2). Compound **4** was identified as luteolin 7-β-D-glucoside, a known sage flavone, by comparison of its NMR data with those published (Wang et al., 1998). The NMR spectra of the major flavonoids **5** and **6** were similar to those of **4**, the only difference being the presence of a set of glucuronic acid carbon signals in **5** and **6** instead of the glucose in **4**. The position of attachment of glucuronic acid to luteolin was determined by HMBC experiments which

showed long range coupling between the anomeric H-1″ (δ 5.1) and the C-7 (δ 163.2) in **5** and between H-1″ (δ 4.9) and C-3′ (δ 146.2) in **6**. Thus, **5** was luteolin 7-*O*-β-D-glucuronide and **6** was luteolin 3′-*O*-β-D-glucuronide. While flavone glucosides have been described in sage (Ulubelen et al., 1979; Ulubelen and Miski, 1981; Zarzuelo et al., 1995; Wang et al., 1998), the corresponding glucuronides were relatively unknown and the luteolin 7- and 3′-*O*-glucuronides were the first examples of such flavonoids from sage.

Compound **7** was identified as 6-hydroxyluteolin 7-*O*-β-D-glucoside which had been previously reported in *S. tomentosa* without NMR data presentation (Ulubelen and Miski, 1981). Its ^{13}C NMR spectrum displayed C-5, C-7 and C-9 in the upfield regions at δ 146.7, 151.7, and 149.4, respectively, which agreed with a 5,6,7-trihydroxylated A-ring of flavones (Horie et al., 1998). The linkage between the sugar H-1″ (δ 5.0) and the C-7 position (δ 151.7) of the flavone was established by long range coupling. Compound **8** had almost identical NMR data for the aglycone 6-hydroxyluteolin as compared with those in **7** and for the glucuronic acid as in **5** and **6**, thus **8** was 6-hydroxyluteolin 7-*O*-β-D-glucuronide, a new sage constituent.

Compound **9** was 6,8-di-*C*-β-D-glucopyranosylapigenin, more commonly known as vicenin-2. The carbon signals of two glucose molecules were well resolved when the NMR was performed at elevated temperature (90°C). The *C*-bonds between sugars and the aglycone were revealed by the correlations between two anomeric protons (δ 4.8 and 4.9) and two carbon signals at δ 74.3 and 74.5 in the 2D H,C NMR spectrum (HSQC). The HMBC experiments of **9** further showed that the anomeric proton at δ 4.8 was long range coupled with the C-7 at δ 162.8 and C-5 at δ 159.6 while the other at δ 4.9 was long range coupled with the C-7 and C-9 (δ 155.2). *C*-glycosylated apigenins such as 8-*C*-glucosylapigenin (vitexin) and 6-*C*-glucosyl-8-*C*-arabinosylapigenin (schaftoside) have been reported in *S. blepharophylla* (Bisio et al., 1997), but 6,8-di-*C*-glucosylapigenin was reported here as a sage component for the first time. 6,8-Di-C-β-D-glucosylapigenin was also found in the leaves of *Allophyllus edulis* (Hoffmann-Bohm et al., 1992).

3. Experimental

3.1. Extraction and isolation

The sage (*S. officinalis*) residue (50 g) (recovered from supercritical CO_2 extraction) was extracted with 70% aq. acetone (3×500 ml). The extracts were combined, concentrated and the residue freeze-dried to yield 11 g of solid extract (22%). The extract was fractionated on an HP20 column (20×6 cm) into water and methanol fractions and the glycosides were isolated from the water fraction by column chromatography on Sephadex and/or MCI HP20 using water or aq. methanol (up to 30% methanol). Fractions were collected using an automatic fraction collector and monitored by HPLC. Chromatographically pure compounds were pooled, concentrated and freeze-dried. Yields: 8 mg of **1**, 8 mg of **2**, 6 mg of **3**, 55 mg of **4**, 156 mg of **5**, 80 mg of **6**, 27 mg of **7**, 17 mg of **8** and 21 mg of **9**.

3.2. NMR identification and HPLC analysis

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 300 instrument and chemical shifts (δ) were referenced to solvent signal. HPLC analysis was performed on a Hewlett Packard series 1100 equipped with a DAD detector (set at 280 and 350 nm) and a LiChrospher (®) 100 RP-18 (5 μm) column (125×4 mm) held at 30°C with the following solvent program: solvent A, 2% HOAc in H_2O; solvent B, 2% HOAc in CH_3CN; starting from 4% B up to 12% B in 20 min, to 20% B in 30 min and to 50% B in 45 min. Flow rate was set 1 ml/min.

3.3. trans-p-Coumaric acid 4-O-(2′-O-β-D-apiofuranosyl)-β-D-glucopyranoside (2)

HPLC R_t 10.1 min, on line UV λ_{max} 296 nm. ^{1}H NMR (300 MHz, CD_3OD/D_2O) δ 3.45–4.05 (*m*, sugar-H), 5.10 (*d*, *J* 7.1 Hz, H-1′), 5.44 (*d*, *J* 1.9 Hz, H-1″), 6.41 (*d*, *J* 16.0 Hz, H-8), 7.09 (*d*, *J* 8.7 Hz, H-3, H-5), 7.40 (*d*, *J* 16.0 Hz, H-7), 7.55 (*d*, *J* 8.7 Hz, H-2, H-6). ^{13}C NMR (75 MHz, CD_3OD/D_2O) see Table 1.

3.4. cis-p-Coumaric acid 4-O-(2′-O-β-D-apiofuranosyl)-β-D-glucopyranoside (3)

HPLC R_t 11.7 min, on line UV λ_{max} 282 nm. ^{1}H NMR (300 MHz, D_2O) δ 3.2–4.0 (*m*, sugar-H), 5.19 (*d*, *J* 7.3 Hz, H-1′), 5.38 (*d*, *J* 2.1 Hz, H-1″), 5.99 (*d*, *J* 12.6 Hz, H-8), 6.44 (*d*, *J* 12.6 Hz, H-7), 7.04 (*d*, *J* 8.6 Hz, H-3, H-5), 7.41 (*d*, *J* 8.6 Hz, H-2, H-6). ^{13}C NMR (75 MHz, D_2O) see Table 1.

3.5. Luteolin 7-O-glucuronide (5)

HPLC R_t 26.1 min, on line UV λ_{max} 254, 350 nm. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.17–3.41 (*m*, sugar-H), 3.60 (*d*, *J* 9.8 Hz, H-5″), 5.07 (*d*, *J* 7.2 Hz, H-1″), 6.41 (*d*, *J* 2.0 Hz, H-6), 6.66 (*s*, H-3), 6.74 (*d*, *J* 8.0 Hz, H-5′), 6.76 (*d*, *J* 2.2 Hz, H-8), 7.34 (*d*, *J* 2.0 Hz, H-2′), 7.35 (*dd*, *J* 2.0, 8.0 Hz, H-6′). ^{13}C NMR (75 MHz, DMSO-d_6) see Table 2.

3.6. Luteolin 3′-O-glucuronide (6)

HPLC R_t 31.8 min, on line UV λ_{max} 238, 268, 342 nm. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.17–3.46 (*m*, sugar-

Table 2
^{13}C NMR data of flavone glycosides **4–9**

C	4	5	6	7	8	9
2	164.91	165.45	163.65	164.64	164.70	164.13
3	103.36	101.97	103.22	102.90	102.57	102.86
4	182.20	181.88	181.96	182.62	182.58	182.30
5	161.47	161.37	161.65	146.97	147.01	159.58
6	99.88	99.83	99.25	130.82	130.93	108.50
7	163.27	163.20	164.99	151.67	151.80	162.78
8	95.09	94.91	94.57	94.37	94.52	104.78
9	157.29	157.22	157.60	149.38	149.32	155.23
10	105.69	105.51	103.78	106.19	106.17	103.60
1′	121.37	118.36	121.44	122.02	121.53	122.01
2′	113.77	112.69	116.32	113.84	113.80	128.93
3′	146.29	147.38	146.21	146.12	146.35	116.27
4′	150.81	154.00	152.39	150.10	150.64	161.42
5′	116.37	116.49	117.14	116.33	116.57	116.27
6′	119.55	119.89	122.53	119.32	119.18	128.93
1″	100.28	100.10	102.97	101.34	101.37	74.32/74.51
2″	73.48	73.36	73.60	73.56	73.36	71.87/71.87
3″	76.75	76.88	76.40	77.64	76.14	78.76/79.10
4″	69.93	72.31	72.63	70.05	72.40	70.32/70.85
5″	77.51	74.09	74.42	76.18	74.51	81.41/81.88
6″	60.99	172.21	172.16	61.04	172.48	61.07/61.53

H), 3.56 (*d*, *J* 9.0 Hz, H-5″), 4.86 (*d*, *J* 7.0 Hz, H-1″), 6.15 (*d*, *J* 2.1 Hz, H-6), 6.50 (*d*, *J* 2.1 Hz, H-8), 6.73 (*s*, H-3), 6.93 (*d*, *J* 8.5 Hz, H-5′), 7.60 (*dd*, *J* 2.1, 8.5 Hz, H-6′), 7.82 (*d*, *J* 2.1 Hz, H-2′). ^{13}C NMR (75 MHz, DMSO-d_6) see Table 2.

3.7. 6-Hydroxyluteolin 7-O-glucoside (*7*)

HPLC R_t 23.3 min, on line UV λ_{max} 232, 282, 344 nm. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.22–3.44 (*m*, sugar-H), 3.76 (*d*, *J* 10.1 Hz, H-6″), 5.01 (*d*, *J* 5.6 Hz, H-1″), 6.69 (*s*, H-3), 6.90 (*d*, *J* 6.8 Hz, H-5′), 6.97 (*s*, H-8), 7.40 (*s*, H-2′), 7.41 (*d*, *J* 7.0 Hz, H-6′), 12.73 (*s*, 5-OH). ^{13}C NMR (75 MHz, DMSO-d_6) see Table 2.

3.8. 6-Hydroxyluteolin 7-O-glucuronide (*8*)

HPLC R_t 22.5 min, on line UV λ_{max} 232, 282, 344 nm. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.26–3.40 (*m*, sugar-H), 3.67 (*d*, *J* 9.5 Hz, H-5″), 5.00 (*d*, *J* 7.1 Hz, H-1″), 6.65 (*s*, H-3), 6.88 (*d*, *J* 8.4 Hz, H-5′), 6.95 (*s*, H-8), 7.35 (*dd*, *J* 8.4, 2.2 Hz, H-6′), 7.42 (*d*, *J* 2.2 Hz, H-2′). ^{13}C NMR (75 MHz, DMSO-d_6) see Table 2.

3.9. 6,8-Di-C-glucosylapigenin or vicenin-2 (*9*)

HPLC R_t 17.6 min, on line UV λ_{max} 232, 270, 336 nm. ^{1}H NMR (500 MHz, DMSO-d_6, 90°C) δ 3.30 (*m*, H-5″), 3.32 (*m*, H-3″, H-4″), 3.35 (*m*, H-5‴), 3.38 (*m*, H-3‴), 3.42 (*m*, H-4‴), 3.58 (*dd*, *J* 11.9, 7.8 Hz, H-6a″, H-6a‴), 3.70 (*d*, *J* 11.0 Hz, H-6b″), 3.76 (*d*, *J* 11.0 Hz, H-6b‴), 3.86 (*t*, *J* 8.7 Hz, H-2″, H-2‴), 4.76 (*d*, *J* 9.8 Hz, H-1″), 4.88 (*d*, *J* 9.8 Hz, H-1‴), 6.59 (*s*, H-3), 6.91 (*d*, *J* 8.5 Hz, H-3′, H-5′), 7.92 (*d*, *J* 8.7 Hz, H-2′, H-6′). ^{13}C NMR (DMSO-d_6, 125 MHz, 90°C) see Table 2.

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