PERGAMON

Phytochemistry 52 (1999) 1149–1152

PHYTOCHEMISTRY

Sagecoumarin, a novel caffeic acid trimer from *Salvia officinalis*

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Received 28 April 1999; accepted 25 June 1999

Abstract

Three structurally related caffeic acid trimers, melitric acid A, methyl melitrate A and the novel sagecoumarin, were isolated from sage and their chemical structures elucidated by means of NMR spectroscopy and mass spectroscopy. The mechanism for their formation from salvianolic acid K was proposed. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Salvia officinalis*; Labiatae; Caffeic acid trimer; Melitric acid A; Methyl melitrate A; Sagecoumarin

1. Introduction

Sage is a popular herb which is widely cultivated in various parts of the world. In New Zealand sage is cultivated mainly for its essential oil and antioxidant ingredients. This antioxidant effect has been attributed to the main phenolic components, rosmarinic acid, a caffeic acid dimer, and carnosic acid (Brieskorn & Dömling, 1969; Chang, Ostric-Matijasevic, Hsieh & Huang, 1977; Cuvelier, Berset & Richard, 1994). Sage also has been shown to contain an impressive array of phenolic compounds derived from caffeic acid such as the trimers, tetramers and higher oligomers including salvianolic acids, lithospermic acids and yunnaneic acids (Ai & Li, 1988, 1992; Tanaka et al., 1989; Tanaka, Nishimura, Kouno, Nonaka & Young, 1996; Tanaka, Nishimura, Kouno, Nonaka & Yang, 1997; Tezuka et al., 1998; Zhang & Li, 1994). The presence of such an interesting variety of potentially valuable bioactive compounds in sage prompted us to conduct a chemical investigation of the residues left from supercritical carbon dioxide extraction of the herb. We earlier reported the isolation and identification of salvianolic acid K (a caffeic acid trimer) and sagerinic acid (a caffeic acid tetramer) along with the main compound rosmarinic acid from the sage residue (Lu & Foo, 1999). This report is a continuation of this study and deals with the separation and structure elucidation of three further caffeic acid derived products from the methanol extract of the sage residue.

2. Results and discussion

HPLC analysis of the methanol extract of sage residue showed that the extract contained rosmarinic acid as the major compound together with several minor components, which were separated by column chromatography on Sephadex LH20 followed by MCI-HP20. This resulted in the isolation of three caffeic acid trimers, namely melitric acid A (**1**), the methyl ester (**2**) and the novel compound, sagecoumarin (**3**).

The NMR spectral data for compound **1** were in agreement with those published for melitric acid A reported in *Melissa officinalis* (Agata, Kusakabe, Hatano, Nishibe & Okuda, 1993), also known as salvianolic acid I as it was independently identified in *Salvia cavaleriei* (Zhang & Li, 1994). The upfield-shift of C-7″ (by 1–2 ppm to 127) and the downfield-shift of C-8″ (by ca 4 ppm to 143) of the caffeic acid segment, as compared with published data, suggested that the melitric acid A isolated here existed as a salt form (Kelly, Harruff & Carmack, 1976), comparable to magnesium and potassium-ammonium salts of lithospermic acid (Tanaka et al., 1989). The full assignment

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PII: S0031-9422(99)00368-4

Table 1
^{1}H NMR (300 MHz, CD_3OD) data (δ in ppm) of compounds **1**–**3**

No.	**1**	**2**	**3**[a]
2	7.16 *br s*	7.21 *d* (2.0)	7.20 *d* (1.8)
5	6.78 *d* (8.7)	6.70 *d* (8.3)	7.01 *d* (8.3)
6	6.91 *dd* (8.7)	6.94 *dd* (8.4, 1.4)	7.09 *dd* (8.3, 1.8)
7	7.52 *d* (15.9)	7.52 *d* (15.9)	7.61 *d* (16.0)
8	6.32 *d* (15.9)	6.36 *d* (15.9)	6.43 *d* (16.0)
2′	6.76 *s*	6.81 *d* (1.7)	6.78 *d* (1.8)
5′	6.69 *d* (8.0)	6.73 *d* (8.0)	6.71 *d* (8.0)
6′	6.63 *d* (8.1)	6.68 *dd* (8.0, 1.7)	6.63 *dd* (8.0, 1.8)
7a′	2.98 *dd* (13.4, 8.6)	2.94 *dd* (14.2, 9.5)	3.01 *dd* (14.2, 8.6)
7b′	3.12 *d* (12.6)	3.12 *dd* (14.2, 2.8)	3.13 *dd* (14.2, 3.7)
8′	5.14 *br s*	5.10 *dd* (9.5, 2.8)	5.20 *dd* (8.6, 3.7)
2″	7.24 *br s*	7.27 *d* (2.0)	6.84 *s*
5″	6.73 *d* (8.3)	6.79 *d* (8.3)	6.80 *s*
6″	7.04 *d* (8.2)	7.10 *dd* (8.4, 2.0)	–
7″	7.25 *br s*	7.34 *s*	7.16 *s*
OCH_3	–	3.78 *s*	–

[a] Numbering of the coumarin unit as cinnamic acid system in accord with that in **1** and **2**.

of ^{1}H and ^{13}C chemical shifts of melitric acid A established by 2D NMR techniques are given in Tables 1 and 2. Assignment of the chemical structure of compound **1** to melitric acid A was also supported by electrospray mass spectroscopy (ES-MS) which showed a base peak at m/z 537 for the expected $[M-H]^-$ ion and a peak at m/z 268 corresponding to $[M-2H]^{2-}$ as a result of double ionization. The optical rotation $[\alpha]_D^{20}+41^\circ$ for melitric acid A was consistent with the value $[\alpha]_D^{23}+45^\circ$ reported for the compound by Agata et al. (1993).

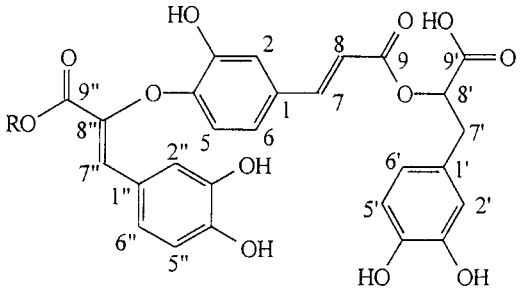
Table 2
^{13}C NMR (75 MHz, CD_3OD) data (δ in ppm) of compounds **1**–**3**

No.	**1**	**2**	**3**[a]
1	131.02	130.43	133.53
2	117.01	116.98	117.76
3	148.72	148.29	150.14
4	149.15	148.29	146.80
5	116.48	115.80	121.34
6	122.65	122.80	122.44
7	146.77	146.68	146.64
8	117.34	118.20	118.27
9	169.11	169.52	168.44
1′	131.05	131.48	130.04
2′	118.00	117.79	117.97
3′	146.43	146.20	146.53
4′	145.36	145.07	145.59
5′	116.68	116.98	116.68
6′	122.21	122.49	122.20
7′	38.87	38.96	38.47
8′	77.41	78.02	75.95
9′	–	–	–
1″	126.95	126.00	112.87
2″	118.27	118.91	112.87
3″	146.65	146.68	145.25
4″	148.41	149.30	150.48
5″	116.68	116.98	103.93
6″	124.74	125.74	147.46
7″	126.99	130.43	125.89
8″	143.21	138.68	140.58
9″	–	166.52	160.46
OCH_3	–	53.67	–

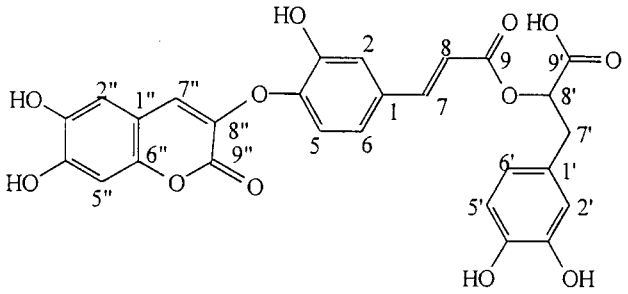
[a] Numbering of the coumarin unit as cinnamic acid system in accord with that in **1** and **2**.

The structure of compound **2** was readily apparent as a methyl ester of melitric acid A from the UV spectrum as well as from its ^{1}H and ^{13}C NMR spectra (Tables 1 and 2). The only difference between the NMR spectra of **2** and that of melitric acid A was the appearance of a three-proton singlet at δ 3.78 and its correlated carbon signal at δ 53.67 in the 2D H,C-COSY spectrum, suggesting that a methyl ester group was present. The strong NOESY effect observed between the methyl ester group and H-7″ (δ 7.34) of the caffeic acid moiety suggested the position of the caffeic acid methyl ester and that the double bond was in a *Z*-configuration as well. Both ^{1}H and ^{13}C NMR data of melitric acid A methyl ester were in good agreement with that of its ethyl ester analogue which is also known as ethyl melitrate A (Agata et al., 1993). Its ES-MS showed a base peak at m/z 551 consistent with the $[M-H]^-$ ion expected for the molecular formula $C_{28}H_{24}O_{12}$. Methyl melitrate A had also been isolated from *Schizonepeta tenuifolia* as an antiinflammatory agent (Matsuda, Kanita, Hitomi, Saito & Yumioka, 1989), its presence in sage had not been detected prior to this study.

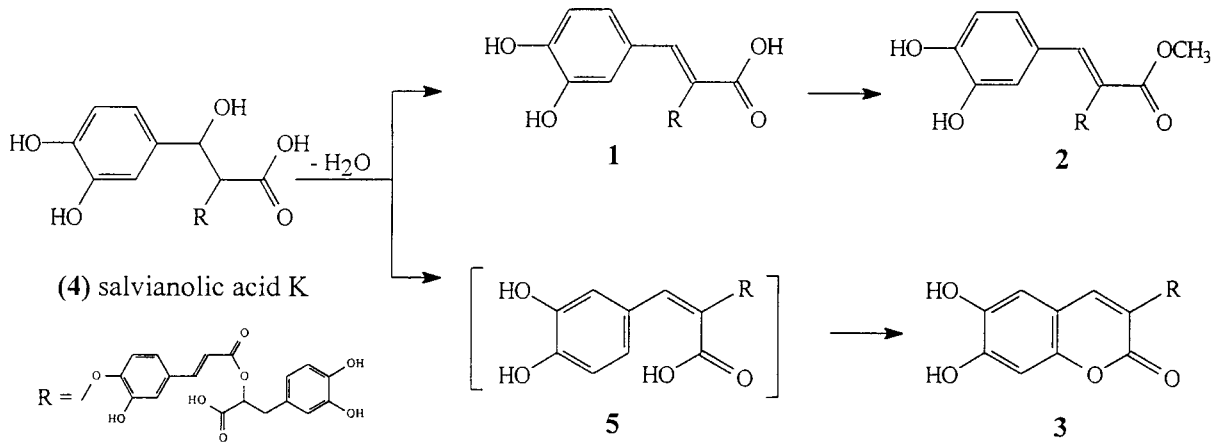
The ^{1}H NMR spectrum of sagecoumarin (**3**) showed the presence of a rosmarinic acid moiety, characterized by two ABX spin systems in the aromatic region for two 3,4-dihydroxyphenyl units, two olefinic protons for a *trans*-configured double bond (δ 7.61 and 6.43, J 16.0 Hz) and three doublets of doublets in the aliphatic region (δ, 5.20; J 8.6, 3.7 Hz; 3.13; J 14.2, 3.7 Hz; 3.01; J 14.2, 8.6 Hz) for a –CH(O–)–CH_2– unit. The ^{13}C NMR spectrum of **3** contained a carboxyl ester (δ 168.44), four phenoxyl carbons (δ 145–150), two quaternary carbons (δ 130.04, 133.53), six aromatic methine carbons (δ 116–122) and two aliphatic carbons (δ 38.47, 75.95), which were consistent with a rosmarinic acid moiety. The ES-MS of **3** showed a base ion peak at m/z 535 which was two hydrogen atoms less than that of melitric acid A, suggesting that some form of intramolecular oxidative cyclization had occurred. In addition to the chemical shifts of the rosmarinic acid functionality the ^{13}C NMR spectrum of **3** contained nine additional carbon signals (a carboxylic ester, four phenoxyl, a quaternary and three tertiary carbons) consistent with a coumarin structure. The three extra singlets at δ 6.80, 6.84 and 7.16 observed in the ^{1}H NMR spectrum suggested that the coumarin structure was 3 (or 4), 6,7-trisubstituted. The linkage between the C(4)-OH of the rosmarinic



(1) R=H melitric acid A
(2) R=CH_3 methyl melitrate A



(3) sagecoumarin



(4) salvianolic acid K

Scheme 1. Proposed mechanism of the formation of melitric acid A (**1**), its methyl ester (**2**) and sagecoumarin (**3**) from salvianolic acid K (**4**).

acid unit and the C-3 position of the coumarin unit via an ether bond was deduced from melitric acid A and confirmed by NOESY experiment which showed positive interaction between H-7″ (δ 7.16) of the coumarin and H-5 (δ 7.01) of the rosmarinic acid moieties. The full assignment of the ^{1}H and ^{13}C NMR resonances of sagecoumarin (Tables 1 and 2) was made using HMQC and HMBC experiments. Sagecoumarin was therefore a novel coumarin compound linked via an ether bond on its C-3 position to a rosmarinic acid.

Melitric acid A, its methyl ester and sagecoumarin could be derived from salvianolic acid K (**4**), a compound previously isolated from the water fraction of sage extract (Lu & Foo, 1999). As shown in Scheme 1, the β-(3,4-dihydroxyphenyl)glyceric acid moiety of salvianolic acid K could readily undergo dehydration to form *Z*- and *E*-caffeic acids **1** and **5**, the former can be esterified to give the methyl ester **2** while the latter oxidatively cyclized to the coumarin **3**.

3. Experimental

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 300 instrument and chemical shifts (δ) were referenced to the solvent signal. HPLC was performed as described previously (Lu & Foo, 1999).

3.1. Isolation of caffeic acid trimers

Sage residue from supercritical CO_2 extraction of ground *S. officinalis* (500 g) was extracted with 70% aqueous acetone (2 l × 3) and the conc. aq. extract first partitioned into a water fraction (36 g) and a methanol fraction (41 g) by chromatography on an HP20 column (180 × 60 cm). The methanol fraction (30 g) was further fractionated on a Sephadex LH20 column (60 × 4 cm) into subfraction I (25 g) containing predominantly rosmarinic acid with up to 40% methanol and subfraction II (2 g) (40–80% methanol). The latter fraction was then chromatographed on a MCI-HP20 column (50 × 2.5 cm) with aq. ethanol, the proportion of ethanol gradually increasing from 50 to 80%. Fractions were collected in 20-ml volumes and monitored by HPLC. Fractions containing the same pure compounds were combined, concentrated on a rotary evaporator and the residue freeze-dried.

3.1.1. Melitric acid A (*1*)

Freeze-dried light-brown powder (119 mg). HPLC

R_t 35.0 min $[\alpha]_D^{20}+41^\circ$ (MeOH *c* 0.2). ES-MS (negative mode) *m/z* (rel. int.): 537.2 ($[M-H]^-$, 100), 358.2 ([M−caffeic acid]$^-$, 15), 268.5 ($[M-2H]^{2-}$, 40). UV λ_{nm}^{MeOH} nm (log ϵ): 290 (4.32), 328 (4.34). ^{1}H and ^{13}C NMR: Tables 1 and 2.

3.1.2. Methyl melitrate A (**2**)

Freeze-dried light-brown powder (60 mg). HPLC R_t 38.9 min $[\alpha]_D^{20}+40.5^\circ$ (MeOH *c* 0.2). ES-MS (negative mode) *m/z* (rel. int.): 551.3 ($[M-H]^-$, 100). UV λ_{max}^{MeOH} nm (log ϵ): 292 (4.33), 330 (4.36). ^{1}H and ^{13}C NMR: Tables 1 and 2.

3.1.3. Sagecoumarin (**3**)

Freeze-dried light-brown powder (23 mg). HPLC R_t 37.0 min. $[\alpha]_D^{20}+52^\circ$ (MeOH *c* 0.2). ES-MS (negative mode) *m/z* (rel. int.): 535.3 ($[M-H]^-$, 100). UV λ_{max}^{MeOH} nm (log ϵ): 288 (4.30), 332 (4.27). ^{1}H and ^{13}C NMR: Tables 1 and 2.

Acknowledgements

The authors wish to acknowledge the assistance of Ms Wendy Jackson of the University of Waikato for providing the ES-MS data.

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