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Rosmarinic acid derivatives from *Salvia officinalis*

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Abstract

Sagerinic acid, a novel cyclobutane and salvianolic acid K, derived from rosmarinic acid, were isolated together with the parent compound from polar solvent extracts of *Salvia officinalis*. Their chemical structures were elucidated by NMR and, for sagerinic acid, the stereochemistry of the substituents on the cyclobutane moiety was established as 3β,4α-diaryl-1α,2β-dicarboxylic acid diester (μ-truxinate form).

Keywords: Salvia officinalis; Labiatae; Rosmarinic acid; Salvianolic acid K; Sagerinic acid; μ-truxinate

1. Introduction

Sage (*Salvia*) is a popular herb which has been used in a variety of food preparations since ancient times. Judging from its Latin name, sage has clearly long enjoyed a reputation in folklore for its health giving properties and for treating all kinds of ailments (Keller, 1978). Although today the most widespread use of the herb is in flavouring foods, it is still commonly used as a household remedy, mainly as an aid in drying up the flow of mother's milk, in reducing saliva secretion, as an anhidrotic to control night sweats associated with illness and in relieving oral cavity and throat inflammations (Steinegger & Hänsel, 1988). Sage has been observed to have excellent properties in inhibiting lipid peroxidation and this activity is attributed principally to the presence of phenolic compounds, such as carnosic acid, carnosol and rosmarinic acid (Zhang, Bao, Wu, Rosen, & Ho, 1990; Cuvelier, Berset, & Richard, 1994; Cuvelier, Richard, & Berset, 1996). Our interest in sage was prompted by its history of health properties and enhanced by recent studies (Ai & Li, 1988; Tanaka et al., 1989; Tanaka, Nishimura, Kouno, Nonaka, & Young, 1996; Tanaka, Nishimura, Kouno, Nonaka, & Yang, 1997), which revealed the presence of some unusual compounds that could be recovered after the volatile flavouring ingredients had been removed.

2. Results and discussion

Column chromatography of sage extract on Sephadex LH20 yielded rosmarinic acid (**1**) as the main compound. Its identity was evident from its ^{1}H and ^{13}C NMR spectra which were consistent with published data (Kelly, Harruff, & Carmack, 1976; Eicher, Ott, & Speicher, 1996). Also isolated were two other compounds, **2** and **3**. The ^{1}H NMR spectrum of compound **2** showed two doublets at δ 7.32 and 6.14 which on the basis of the observed large proton-proton coupling (J 16.0 Hz) were assigned to a pair of *trans*-olefinic protons. In addition, there were three ABX-spin systems (Table 1) observed in the aromatic region, which were assignable to the three discrete sets of protons of the 3,4-dihydroxyphenyl unit. Also observed were two sets of doublets at δ 4.70 and 5.09 ($J = 5.1$ Hz) in the low field region, suggesting two neighbouring methine protons attached to oxygen-bearing carbons and a further three sets of multiplets at δ 3.00, 3.14 and 5.05 attributable to three protons coupled in an ABX pattern ($J = 14.2$, 9.1 and 3.8 Hz), consistent with the presence of a –CH(OH)–CH_2– unit. The ^{13}C NMR spectrum of **2** (Table 2) showed the presence of three carbonyl carbons, of which two were identifiable with those of carboxylic acids (δ 179.9 and 178.6) and one of a carboxyl ester (δ 171.4). The presence of three sets of 3,4-dihydroxyphenyl groups was confirmed by the appearance of 18 aromatic carbons consisting of nine quaternary carbons, of which six were phenoxyl carbons (δ 146–151) and nine tertiary carbons (δ 117–125). In addition, there were also two olefinic carbons (δ 148.2

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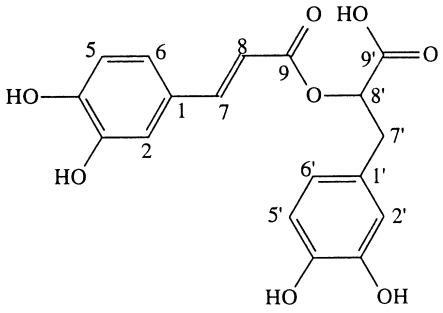
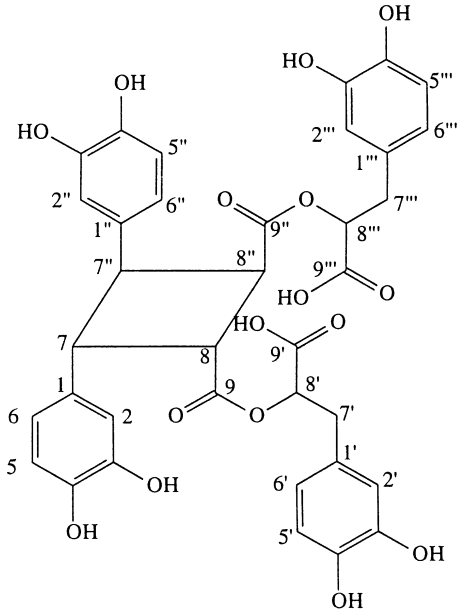
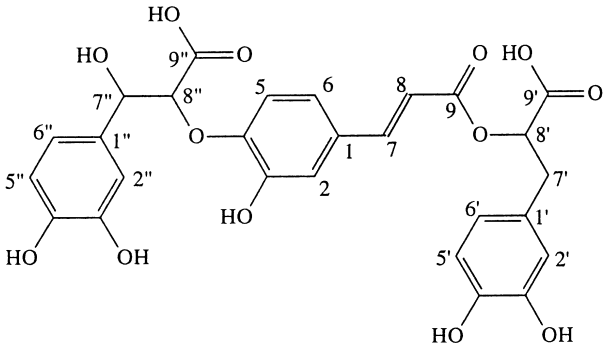


(**1**) Rosmarinic acid



(**3**) Sagerinic acid



(**2**) Salvianolic acid K

and 118.0), three oxygenated methine carbons (δ 86.7, 79.2 and 76.4) and one methylene carbon (δ 39.6) present in the spectrum. These chemical shifts, corroborated by ^{1}H-^{1}H and ^{1}H-^{13}C COSY studies, were consistent with the presence of a rosmarinic acid and a β-(3,4-dihydroxyphenyl)glyceric acid moieties in the structure of **2**. The manner in which the rosmarinic acid and β-(3,4-dihydroxyphenyl)glyceric acid were linked together in **2** was established by application of a long-range coupling experiment (HMBC) which showed coupling between the H-8″ (δ 4.7) and C-4 (δ 151.1), hence, the ether linkage was at the α-OH of glyceric acid and the C(4)-OH of the caffeoyl moiety in rosmarinic acid. The full assignments of ^{1}H and ^{13}C chemical shifts of **2** are shown in Tables 1–2, respectively. Its chemical structure was also confirmed by electrospray mass spectroscopy (ESMS) which showed an $[M-H]^{-}$ at m/z 555 and a base peak at m/z 276.8 corresponding to $[M-2H]^{2-}$ due to double ionization. Compound **2** was therefore identical to salvianolic acid K which was recently reported to be present in the roots of Chinese desert sage (*Salvia deserta*) (Tezuka et al., 1998).

The ^{13}C NMR spectrum of sagerinic acid (**3**) (Table 2) showed some similarity to that of rosmarinic acid, except that there were twice as many carbon signals present as those observed for rosmarinic acid. The eight phenoxyl carbon signals (δ 145–146), 12 tertiary carbon signals (δ 117–124) and four quaternary carbon signals (δ 132–133) (established using DEPT) were consistent with the presence of four sets of 3,4-dihydroxyphenyl groups. This was corroborated by the ^{1}H NMR spectrum (Table 1) which showed 12 aromatic protons (δ 6.5–7.1) coupled in manner consistent with four discrete ABX-resonance systems. The ^{1}H NMR spectrum also showed couplings between the protons at δ 2.5–2.9 and those at δ 4.3–4.4, these protons being associated with the corresponding four aliphatic carbons at δ 37.37, 38.99, 79.48 and 79.77, respectively, as shown by HMQC, thus indicating the presence of two –CH(OH)–CH_2– moieties. Sagerinic acid differed from rosmarinic acid in the absence of the olefinic

Table 1
^{1}H NMR (300 MHz, D_2O) data for rosmarinic acid (**1**), salvianolic acid K (**2**) and sagerinic acid (**3**)

No.	**1**[a]	**2**	**3**
2	7.17 d (1.9)	6.91 s	6.56 d (2.1)
5	6.88 d (8.8)	6.67 d (8.4)	6.58 d (8.2)
6	7.00 dd (8.8, 2.0)	6.75 d (8/4)	5.99 dd (8.2, 2.1)
7	7.53 d (16.0)	7.32 d (16.0)	4.07 dd (10.6, 6.5)
8	6.34 d (16.0)	6.14 d (16.0)	3.60 dd (10.6, 6.5)
2′	6.89 br s	6.91 s	6.70 d (2.0)
5′	6.75 d (8.0)	6.87 d (8.1)	6.72 d (8.1)
6′	6.71 dd (8.0, 1.7)	6.79 d (8.1)	6.59 dd (8.1, 2.0)
7′	2.95 dd (13.8, 8.8)	3.00 dd (14.2, 9.1)	2.71 dd (14.0, 9.7)
	3.16 d (13.8)	3.14 dd (14.2, 3.8)	2.85 dd (14.0, 3.5)
8′	5.07 d (8.8)	5.05 dd (9.1, 3.8)	4.35 dd (9.3, 3.7)
2″		7.07 s	6.73 d (2.2)
5″		6.89 d (8.3)	6.63 d (8.2)
6″		6.92 d (8.3)	6.38 dd (8.3, 2.1)
7″		5.09 d (5.1)	4.08 dd (10.6, 7.8)
8″		4.70 d (5.1)	3.70 dd (10.6, 7.8)
2‴			6.60 d (2.0)
5‴			6.70 d (8.1)
6‴			6.41 dd (8.1, 2.0)
7‴			2.52 dd (14.3, 4.8);
			2.62 dd (14.3, 7.6)
8‴			4.33 dd (7.6, 4.8)

[a] Measured in CD_3OD.

Table 2
^{13}C NMR (75 MHz, D_2O) data for rosmarinic acid (**1**), salvianolic acid K (**2**) and sagerinic acid (**3**)

Carbon	**1**[a]	**2**	**3**
1	129.5	131.2	133.6
2	117.3	118.0	118.5
3	143.4	148.6	146.1
4	145.6	151.1	145.4
5	118.5	118.1	118.8
6	124.1	124.8	120.9
7	148.7	148.2	43.7
8	117.0	118.0	50.1
9	171.4	171.4	176.5
1′	133.1	133.0	132.7
2′	119.6	119.9	119.5
3′	146.8	146.5	146.5
4′	147.5	145.3	145.3
5′	118.7	118.6	118.6
6′	125.2	124.7	124.6
7′	39.9	39.6	39.4
8′	79.3	79.2	79.8
9′	179.5	179.9	179.2
1″		134.8	133.8
2″		117.9	117.6
3″		146.5	146.2
4″		146.4	145.6
5″		118.9	118.7
6″		122.7	122.8
7″		76.4	43.0
8″		86.7	48.9
9″		178.6	175.9
1‴			132.3
2‴			119.7
3‴			146.4
4‴			145.1
5‴			118.7
6‴			124.3
7‴			39.0
8‴			79.5
9‴			179.1

[a] Measured in CD_3OD.

carbons, which were replaced by four tertiary aliphatic carbons at δ 43.03, 43.71, 48.91 and 50.10 (established by DEPT). The methine protons at δ 3.6–3.7 and 4.0–4.1, associated with these carbons were mutually coupled to one another and, therefore, were covalently linked in a cyclobutane ring. Thus, sagerinic acid was a novel rosmarinic acid dimer, where dimerization had occurred by a [2+2] union of the olefinic moieties. The cyclobutane structure of sagerinic acid was further supported by ESMS using negative ion detection, which gave a prominent parent ion at m/z 719 consistent with $[M–H]^-$ and a base peak at m/z 359 corresponding to $[M–2H]^{2-}$.

The complete assignment of the ^{1}H and ^{13}C NMR chemical shift data of sagerinic acid was achieved by extensive use of ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and inverse long range ^{1}H, ^{13}C coupling (HMBC) experiments. The configuration of sagerinic acid was established using NOESY which showed positive interactions between H-6 (δ 5.99) and H-7″ (δ 4.08), H-6 and H-8″ (δ 3.70), as well as those between H-6″ (δ 6.38) and H-7 (δ 4.07), H-6″ and H-8 (δ 3.60). Similar NOESY effects were also observed between protons H-6 (δ 5.99) and H-8′ (δ 4.35), H-8′ and H-8″ (δ 3.70), and H-8 (δ, 3.60) and H-8‴ (δ 4.33). These clearly indicated that the substitutents on the cyclobutane ring was in a μ-truxinate arrangement (Green & Rejtö, 1974). Sagerinic acid is possibly formed from rosmarinic acid via a photochemical cyclization process. Similar dimeric products with cyclobutane structures derived from *p*-coumaric acid, ferulic acid and cinnamic acid amides are also known to be present in the cell walls of tropical grasses (Ford & Hartley, 1990; Morrison, Robertson, Stewart, & Wightman, 1991), bamboos (Tachibana, Ohkubo, & Towers, 1992a, 1992b) and *Piper* species (Filho, De Souza, & Mattos, 1981; Duh, Wu, & Wang, 1990; Maxwell & Rampersad, 1991). More recently, stachysetin, an analogous apigenin-7-*p*-coumaroylglucoside cyclodimer in the μ-truxinate form, was reported in the aerial parts of *Stachys aegyptiaca* (El-Ansari, Nawwar, & Saleh, 1995).

3. Experimental

^{1}H and ^{13}C NMR were recorded on a Bruker AC 300 instrument and chemical shifts (δ) were referenced to the solvent signal. HPLC was performed on 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 MeCN; starting from 4 up to 12% B in 20 min, to 20% B in 30 min and to 50% B in 45 min. The flow rate was 1 ml min^{-1} and detection was made at 280 nm.

3.1. Extraction, fractionation and isolation

The residue after supercritical CO_2 extraction of ground *S. officinalis* (50 g) was extracted with 70% aq. Me_2CO (3 × 500 ml) and the combined extracts conc. and freeze-dried to yield 11 g of solid material (22%). The extract (10 g) was fractionated on a HP20 column (20 × 6 cm) into a H_2O fr. (500 ml) and a MeOH fr. (300 ml), which were conc. and freeze-dried to afford 4.5 g (H_2O fr.) and 4.6 g (MeOH fr.), respectively. These fr. were separately chromatographed on a Sephadex LH20 column (50 × 2.5 cm) and eluted with aq. MeOH (up to 30%). Frs. were collected and monitored by HPLC and the sorted frs. conc and freeze-dried. Where necessary frs. were further purified on Sephadex LH20 until chromatographically pure compounds were obtained.

3.2. Rosmarinic acid (*1*)

2530 mg from MeOH fr. HPLC R_t 30.8 min. ^1H and ^{13}C NMR: Tables 1–2.

3.3. Salvianolic acid K (*2*)

288 mg from H_2O fr. HPLC R_t 31.6 min. ES–MS (negative mode) *m/z* (rel. int.) 555 ($[M–H]^-$ 3), 277 ($[M–2H]^{2-}$ 100). UV λ_{max}^{MeOH} nm (log ε), 212 (4.22), 288 (4.15), 322 sh (3.97). ^1H and ^{13}C NMR: Tables 1–2.

3.4. Sagerinic acid (*3*)

65 mg from H_2O fr. HPLC R_t 29.4 min. $[\alpha]_D^{20}$ +4° (MeOH *c* 0.2). ES–MS (negative mode) *m/z* (rel. int.) 719.3 ($[M–H]^-$ 3), 359.2 ($[M–2H]^{2-}$ 100). UV λ_{max}^{MeOH} nm (log ε) 232 (4.08), 286 (4.09). ^1H and ^{13}C NMR: Tables 1–2.

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