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Phytochemistry 63 (2003) 859–862
PHYTOCHEMISTRY
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Diterpenoid from *Salvia greggii*

Nobuo Kawahara[a,*], Mayumi Inoue[a], Ken-ichi Kawai[b], Setsuko Sekita[a], Motoyoshi Satake[c], Yukihiro Goda[a]

[a]*National Institute of Health Sciences (NIHS), Kamiyoga 1-18-1, Setagaya-ku, Tokyo 158-8501, Japan*
[b]*Faculty of Pharmaceutical Sciences, Hoshi University, Ebara 2-4-41, Shinagawa-ku, Tokyo 142-8501, Japan*
[c]*Japan Pharmacists Education Center (JPEC), Toranomon 19 Mori Bldg. Toranomon 1-2-20 Minato-ku, Tokyo 105-0001, Japan*

Received 6 February 2003; received in revised form 12 March 2003

Abstract

The structure of a diterpenoid, designated salvigresin, that was isolated from the aerial parts of *Salvia greggii*, has been confirmed by spectroscopic investigation and X-ray analysis.

Keywords: *Salvia greggii*; Labiatae; Autumn sage; Salvigresin; Diterpenoid; X-ray analysis

1. Introduction

Salvia greggii (Labiatae), a biennial plant originating from both Mexico and the Texas region, is commonly known as "Autumn Sage." In our ongoing research into its chemical constituents a new diterpenoid was isolated, designated salvigresin (**1**), along with salviarin (**2**, Savona et al., 1978), isopimaric acid (**3**, Wenkert and Buckwalter, 1972) and 14α-hydroxy isopimaric acid (**4**, Bruno et al., 1986), from a CH_2Cl_2 extract of the aerial parts. The structural elucidation of compound **1** is reported in this paper.

2. Results and discussion

Salvigresin (**1**) formed colorless prisms and was found to have the molecular formula $C_{24}H_{30}O_8$ by high-resolution time of flight (TOF) mass spectrometry, giving a molecular ion at m/z 469.1830 (calc. 469.1838) $(M+Na)^+$. The IR and the UV spectra indicated the presence of an α, β-unsaturated carbonyl group. The ^1H NMR spectrum of **1** exhibited 29 non-exchangeable protons, including one secondary (δ 1.04) and three tertiary (δ 0.82, 2.08 and 2.11) methyl groups as well as four olefinic protons (δ 6.39, 6.76, 7.36 and 7.41). The ^{13}C NMR spectrum of **1** showed four methyls, four methylenes including an oxygenated carbon (δ 71.3), five methines including three oxygenated carbons (δ 63.4, 66.3 and 73.5), four tertiary and two quaternary carbon atoms assignable to double bonds, and three carbonyl functions (δ 169.91, 169.86 and 168.2). The two methyl groups (δ 2.08 and 2.11) and ester carbonyl groups suggested the presence of two acetoxyl moieties in the molecule. The olefinic carbons and the three carbonyl groups accounted for six of the ten unsaturations, thus implying that **1** consisted of a four-ring system with a structure related to salviarin (**2**).

Interpretation of the ^1H–^1H COSY and PFGHMQC spectra of **1** suggested the presence of five partial structures **A**–**E** (Fig. 1), in addition to two quaternary carbons (δ 38.3 and 44.8), three methyl groups (δ 19.1, 20.9 and 21.3) and the above three carbonyl carbons. The connectivity of these partial structures was deduced from the PFGHMBC spectrum (Fig. 1). The singlet methyl group at δ 0.82 (H_3-20) showed correlations to the quaternary carbon at δ 38.3 (C-9), the methine carbon at δ 40.6 (C-10) of segment **A**, the methine carbon at δ 40.3 (C-8) of segment **B** and the methylene carbon at δ 45.6 (C-11) of segment **D**. The methine proton (δ 2.59, H-10) and the olefinic proton (δ 6.76, H-3) of segment **A**, the methylene protons (δ 1.64 and 2.34, H_2-6) of segment **B** and the methylene protons (δ 3.94 and

* Corresponding author. Tel.: +81-3-3700-9165; fax: +81-3-3707-6950.
E-mail address: kawahara@nihs.go.jp (N. Kawahara).

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doi:10.1016/S0031-9422(03)00216-4

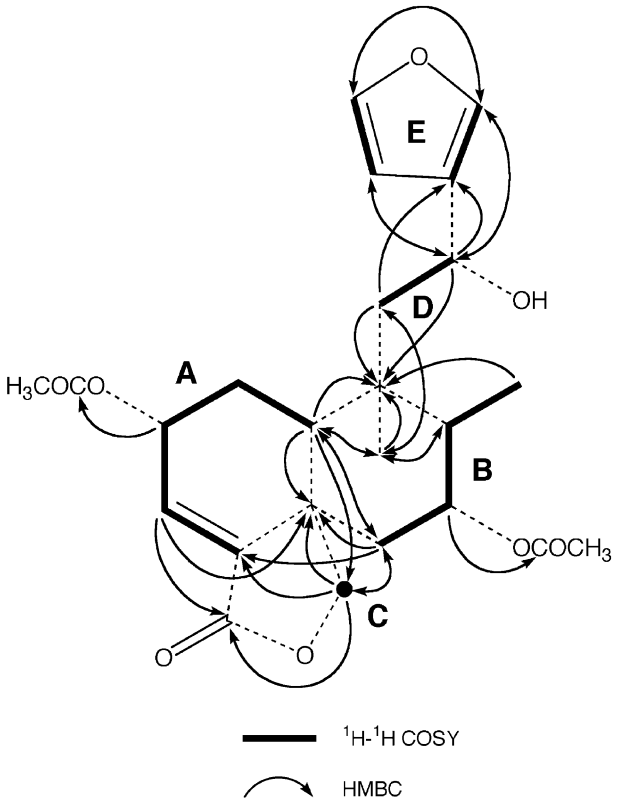


Fig. 1. ^{1}H–^{1}H and major long-range ^{13}C–^{1}H correlations of salvigresin (**1**).

4.90, H_2-19) of segment **C** were both correlated to the quaternary carbon at δ 44.8 (C-5).

On the other hand, the methine proton (δ 4.85, H-12) of segment **D** showed correlations to the olefinic carbons at δ 130.7 (C-13), 108.0 (C-14) and 138.0 (C-16) of segment **E**. The olefinic proton (δ 6.76, H-3) of segment **A** and the methylene protons (δ 3.94 and 4.90, H_2-19) of segment **C** were both correlated to the carbonyl group at δ 168.2 (C-18). The correlations observed between H-2 and carbonyl groups at δ 169.91, H-7 and carbonyl groups at δ 169.86 indicated that two acetoxyl groups were attached at C-2 and C-7, respectively.

These data were agreement with the planar structure of salvigresin, as shown in **1**. The assignments of the ^{1}H and ^{13}C NMR signals are summarized in Table 1.

In order to confirm the exact structure of **1**, X-ray crystallographic analysis was conducted. Crystals of **1** were grown from *n*-hexane–EtOAc (1:1) solution in order to obtain colorless prisms. The molecules of **1** are mainly packed by van der Waals forces, and no hydrogen bonding was observed. The molecular structure of **1** is illustrated in Fig. 2. Based on the obtained data, the relative structure of salvigresin, as shown in **1**, was deduced.

The absolute configuration of **1** was determined by ^{1}H NMR spectroscopic analysis of the (+)-(*R*)-and (−)-(*S*)-α-methoxy-α-trifluoromethylphenyl acetates (MTPA

Table 1
^{1}H and ^{13}C NMR chemical shifts and heteronuclear multiple bond correlations (HMBC) of salvigresin (**1**) in $CDCl_3$

Position		^{1}H[a]	*J* (Hz)	^{13}C[b]	HMBC (^{1}H)[c]
1	(α)	1.46 *ddd*	3.4, 12.8, 14.7	24.9 *t*	3, 10
	(β)	1.88 *br. d*	14.7		
2		5.39 *dd*	3.4, 6.4	66.3 *d*	1, 3, 10
3		6.76 *d*	6.4	128.2 *d*	1, 2
4				144.0 *s*	2, 6, 19
5				44.8 *s*	1, 3, 6, 7, 10, 19
6	(α)	2.34 *dd*	2.1, 14.6	37.5 *t*	7, 10, 18
	(β)	1.64 *ddd*	2.1, 3.7, 14.6		
7		5.37 *ddd*	2.1, 3.7, 4.3	73.5 *d*	6, 17
8		2.67 *dq*	4.3, 7.0	40.3 *d*	6, 10, 11, 17, 20
9				38.3 *s*	7, 8, 10, 11, 12, 17, 20
10		2.59 *br. d*	12.8	40.6 *d*	1, 2, 6, 8, 11, 20
11		1.58 *dd*	4.3, 15.6	45.6 *t*	8, 12, 20
		2.04 *dd*	9.8, 15.6		
12		4.85 *dd*	4.3, 9.8	63.4 *d*	11, 14
13				130.7 *s*	11, 12, 14, 15, 16
14		6.39 *dd*	0.6, 1.5	108.0 *d*	12, 15, 16
15		7.41 *t*	1.5	143.9 *d*	14, 16
16		7.36 *dd*	0.6, 1.5	138.0 *d*	12, 14, 15
17	(3H)	1.04 *d*	7.0	11.7 *q*	8
18				168.2 *s*	3, 19
19		3.94 *dd*	2.1, 8.2	71.0 *t*	6, 10
		4.90 *d*	8.2		
20	(3H)	0.82 *s*		19.1 *q*	8, 10, 11
Ac	(3H)	2.08 *s*		20.9 *q*	
				169.9 *s*	2
Ac	(3H)	2.11 *s*		21.3 *q*	
				169.9 *s*	7

[a] 500 MHz.
[b] 125 MHz.
[c] Protons correlating with carbon resonance (optimized for $^{n}J_{CH} = 6$ Hz).

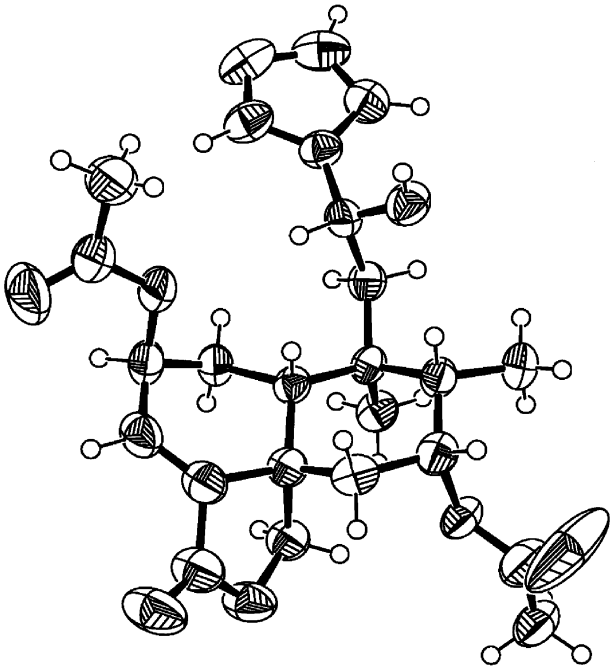
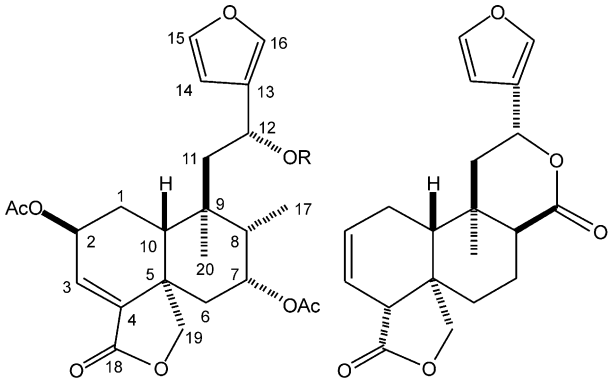


Fig. 2. Perspective view of the crystal structure of salvigresin (**1**).

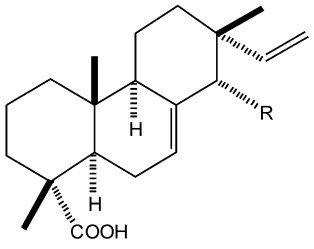
esters) of **1** (namely by the modified Mosher's method, Otani et al., 1991). As shown in Table 2, the ^{1}H NMR signals at 11-H_2, 14-H, 15-H and 16-H of (+)-(*R*)-MTPA ester (**5**) were observed at lower fields than those of (−)-(*S*)-MTPA ester (**6**), while resonances at 1-H_2, 2-H, 6-H_2, 7-H, 8-H, 10-H, 17-Me, 19-H_2, 20-Me of **5** appeared at slightly higher fields as compared to those of **6** (Fig. 3). Consequently, the configuration of C-12 was confirmed to be *R*. From the above results, the structure of salvigresin (**1**) was established as (5*S*, 8*R*, 9*S*, 10*R*)-2*S*, 7*R*-diacetoxy-15, 16-epoxy-12*R*-hydroxycleroda-3, 13(16), 14-trien-18, 19-olide.

A large number of diterpenoids, including **2–4**, have been isolated from the genus *Salvia*, while salvigresin (**1**) is the first example of a diacetylated tetracyclic clerodane diterpenoid. The biological activity of **1** will be investigated in subsequent investigations.



1 : R = H
5 : R = (+)-(*R*)-MTPA
6 : R = (-)-(*S*)-MTPA

2



3 : R = H
4 : R = OH

Table 2
^{1}H NMR chemical shifts of (+)-(*R*)-MTPA ester (**5**) and (−)-(*S*)-MTPA ester (**6**) in $CDCl_3$

Proton[a]		**5**	*J* (Hz)	**6**	*J* (Hz)
1	(α)	1.38 *ddd*	2.9, 12.8, 14.3	1.42 *ddd*	3.3, 11.7, 14.7
	(β)	1.72 *br. dd*	2.9, 14.3	1.79 *br. dd*	3.3, 14.7
2		5.31 *td*	2.9, 6.6	5.34 *td*	3.3, 6.6
3		6.78 *d*	6.6	6.78 *d*	6.6
6	(α)	2.13 *dd*	2.2, 15.0	2.21 *dd*	2.2, 14.7
	(β)	1.17 *ddd*	2.2, 3.7, 15.0	1.34 *ddd*	2.2, 2.6, 14.7
7		4.64 *dt*	2.2, 3.7	4.90 *dt*	2.2, 2.6
8		1.24 *dq*	3.7, 6.6	1.62 *dq*	2.6, 7.0
10		2.24 *br. d*	12.8	2.33 *br. d*	11.7
11		1.54 *d*	16.5	1.53 *d*	16.5
		2.34 *dd*	10.6, 16.5	2.33 *dd*	11.0, 16.5
12		6.03 *d*	10.6	6.01 *d*	11.0
14		6.41 *dd*	0.5, 1.5	6.31 *dd*	0.7, 1.5
15		7.39 *t*	1.5	7.35 *t*	1.5
16		7.45 *dd*	0.5, 1.5	7.39 *dd*	0.7. 1.5
17	(3H)	0.50 *d*	6.6	0.69 *d*	7.0
19		3.84 *dd*	2.2, 8.1	3.87 *dd*	2.2, 8.1
		4.74 *d*	8.1	4.79 *d*	8.1
20	(3H)	0.71 *s*		0.74 *s*	

[a] 600 MHz.

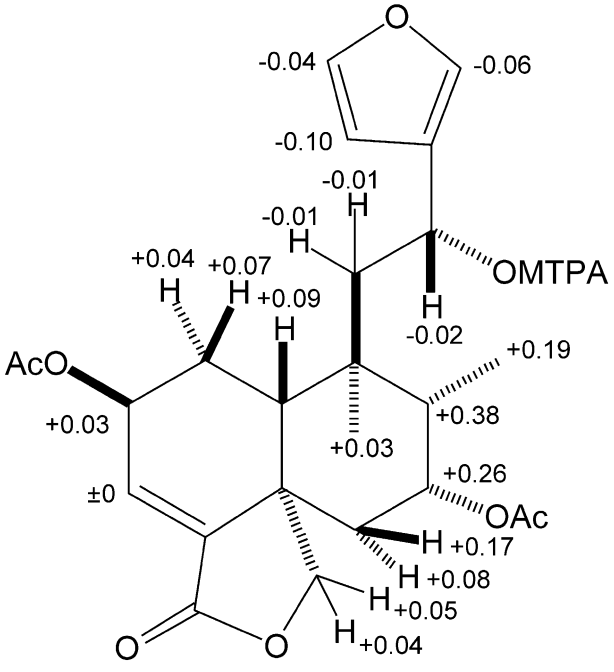


Fig. 3. ^{1}H Chemical shift changes observed in the MTPA ester (**5** and **6**). Values [$\Delta\delta = \delta(S) - \delta(R)$] are given in ppm.

3. Experimental

3.1. General

Mps: uncorr. IR and UV spectra were recorded on JASCO FT/IR-5300 and Shimadzu UV-2550 spectrophotometers, respectively. Optical rotation was measured on a JASCO DIP-370 polarimeter and is given in units of 10^{-1} deg cm^2 g^{-1}. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on Jeol A-600 and A-500 spectrometers, respectively and *J* values are given in Hz. TOF mass (TOF-MS) and high-resolution TOF mass (HR-TOF-MS) spectra were recorded on a Jeol JMS-T100LC spectrometer.

3.2. Plant material

The aerial parts of *Salvia greggii* were cultivated in Wakayama Experimental Station for Medicinal Plants, National Institute of Health Sciences. A voucher specimen (No. SY001) has been deposited at the National Institute of Health Sciences, Japan.

3.3. Extraction and isolation

The aerial parts of *S. greggii* (700 g) were crushed and extracted with MeOH (3 l×3) to give a crude extract (111.5 g), which was partitioned between CH_2Cl_2, EtOAc, *n*-BuOH and H_2O. The CH_2Cl_2-soluble fraction (17.8 g) was separated by activated charcoal CC into three fractions: MeOH, MeOH–$CHCl_3$ (7:3) and $CHCl_3$. Salviarin (**2**, 367 mg) was precipitated from the MeOH fraction and the residue (10.0 g) was subjected to silica gel CC with *n*-hexane–EtOAc (5:1) to afford isopimaric acid (**3**, 70 mg), *n*-hexane–EtOAc (3:1) to afford 14α-hydroxy isopimaric acid (**4**, 1.3 g) and then *n*-hexane–EtOAc (1:1) to afford salvigresin **1** (103 mg).

3.4. Salvigresin (*1*)

Colorless prisms [*n*-hexane–EtOAc (1:1)], mp 242–245 °C, $[\alpha]_D^{25}$ −118.6 °C ($CHCl_3$; c 0.22). IR ν_{max}^{KBr} cm^{-1}: 3457 (OH), 1777 (CO), 1753 (CO) and 1740 (CO). UV $\lambda(\log \epsilon)$: 247 (3.59). HR-TOF-MS (ESI positive) *m*/*z*: 469.1830 $[M+Na]^+$; calc. for $[C_{24}H_{30}O_8+Na]^+$: 469.1838. For ^1H and ^{13}C NMR spectroscopic, see Table 1.

3.5. Preparation of the 2-(+)-(R)-MTPA Ester (*5*) and 2-(−)-(S)- MTPA Ester (*6*)

A solution of salvigresin (**1**) (5.0 mg) in CH_2Cl_2 (0.5 ml) was treated at room temperature (25 °C) with (+)-α-methoxy-α-trifluoromethylphenyl acetic acid (20 mg), dicyclohexylcarbodiimide (DCC) (20 mg), and dimethylaminopyridine (8.0 mg) for 30 min. The reaction mixture was evaporated and the residue was purified by Bond Elute using *n*-hexane–EtOAc (1:1) to give 2-(+)-(*R*)-MTPA ester (**5**) (3.0 mg). In a similar manner, the 2-(−)-(*S*)-MTPA ester (**6**) (3.0 mg) was prepared from **1** (5.0 mg).

3.6. X-Ray structure analysis of salvigresin (*1*)

3.6.1. Structure determination of salvigresin (*1*) by X-ray diffraction

Crystals of **1** were grown from *n*-hexane–EtOAc (1:1) as colorless prisms. The diffraction intensities were collected from a crystal of with dimensions of 0.7×0.3×0.1 mm on a Rigaku AFC-7 FOS four-cycle diffractometer. Of the 2204 reflections (complete for $2\theta < 136.1°$), 2096 satisfied criterion $I > 3\sigma(I)$ and only these were used in the solution and refinement of the structure.

Crystal data, $C_{24}H_{30}O_8$, $M = 446.50$, monoclinic, space group $P2_1$, $a = 10.391(4)$, $b = 14.893$ (9), $c = 7.714(5)$ Å, $\beta = 102.48(4)°$, $V = 1161.4(1)$ $Å^3$, $Z = 2$, $D_c = 1.277$ g cm^{-3}, $F(000) = 476$, Cu-K_α X-radiation (Ni filtered), $\lambda = 1.54178$ Å.

3.6.2. Structure solution and refinement

The structure was solved by direct methods using SIR92 (Altomare et al., 1994), expanded using Fourier techniques (DIRDIF 99, Beurskens et al., 1999) and in the final refinement by the block-matrix least-squares method; anisotropic thermal parameters were used for all non-hydrogen atoms and isotropic thermal parameter for hydrogens were refined. The final refinement converged to R 0.051 (R_w 0.049). A list of atomic parameters, bond lengths, and bond angles will be deposited at the Cambridge Crystallographic Center.

Acknowledgements

The authors are grateful to Mr. Y. Shiga of Wakayama Experimental Station for Medicinal Plants for his assistance in the cultivation of *S. greggii*. This work was supported in part by a grant from the Japan Health Science Foundation.

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