Pergamon

Tetrahedron Letters 42 (2001) 8223–8225

TETRAHEDRON
LETTERS

Salvianolic acid L, a potent phenolic antioxidant from *Salvia officinalis*

Yinrong Lu* and L. Yeap Foo

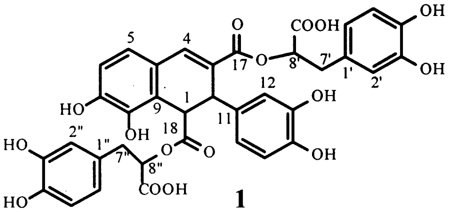
Industrial Research, PO Box 31310, Lower Hutt, New Zealand

Received 7 August 2001; accepted 14 September 2001

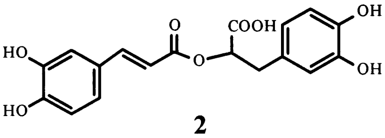
Abstract—Salvianolic acid L, a rosmarinic acid dimer, was isolated from *Salvia officinalis* and identified as 7,8-dihydroxy-2-(3,4-dihydroxyphenyl)-1,2-dihydronaphthalene-1,3-dicarboxylic acid di(1-carboxy-2-(3,4-dihydroxyphenyl))ethyl ester. Two novel hydrolytic products 7,8-dihydroxy-2-(3,4-dihydroxyphenyl)-1,2-dihydronaphthalene-1,3-dicarboxylic acid and the corresponding 3-monoester were also isolated and characterized. Salvianolic acid L showed strong free radical scavenging activities for DPPH and superoxide anion radicals. © 2001 Published by Elsevier Science Ltd.

Sage (*Salvia officinalis*) is a common herbal plant widely cultivated for culinary purposes and its well-known antioxidant properties are attributed mainly to the presence of carnosic acid and rosmarinic acid.[1] Recent studies on sage by Ho and his co-workers[2] revealed the presence of other phenolic compounds that possess moderate antioxidant activities, while our work on New Zealand cultivated sage has led to the identification of several novel compounds[3] which display potent antioxidant activities based on their capacity to scavenge free DPPH (1,1-diphenyl-2-picrylhydrazyl) radical and the xanthine/xanthine oxidase system generated superoxide anion radical.[4] Further examination of the sage extract has led to the isolation of another rosmarinic acid dimer to which we give the name salvianolic acid L (**1**) in line with past practice. This report deals with elucidation of the structure of this compound by NMR spectroscopy and by acid hydrolysis as well as an examination of its antioxidant activity.

Salvianolic acid L was isolated as a brownish powder from a 70% aq. acetone extract of sage by repeated chromatography on Sephadex LH20 column with 50% aq. ethanol. Its ^{13}C NMR chemical shifts[5] showed similarity to those of rosmarinic acid (**2**)[3a] except that there were twice as many carbon signals as those observed for rosmarinic acid. The four carboxylic acid carbon signals were observed at δ 169–178 and in addition there were eight phenoxy carbon signals at δ 144–150, two oxygenated methine carbon signals at δ 78 and two methylene carbon signals at δ 38, all of which suggested salvianolic acid L to be a dimerized rosmarinic acid. The ^{1}H NMR (Table 1), however, showed only three ABX resonance systems in the aromatic region instead of the expected four for catechol moieties. The double bond proton signals were also absent. Two AX_2 resonance systems were observed in the aliphatic region which were consistent with two -CH(O-)-CH$_2$- moieties and two broad singlets at δ 4.28 and 4.45 that were characteristic for H-1 and H-2 in 1,2-dihydronaphthalene moiety as found in rabdosiin[6] and yunnaneic acid G.[7] These clearly suggested that salvianolic acid L was a condensation product of two rosmarinic acid molecules formed via an oxidative cyclization leading to the formation of 1,2-



1



2

Keywords: *Salvia officinalis*; Labiatae; salvianolic acid L; 7,8-dihydroxy-2-(3,4-dihydroxyphenyl)-1,2-dihydronaphthalene-1,3-dicarboxylic acid di(1-carboxy-2-(3,4-dihydroxyphenyl))ethyl ester; antioxidant; hydrolytic products.

* Corresponding author. Fax: +64-4-5690055; e-mail: y.lu@irl.cri.nz

0040-4039/01/$ - see front matter © 2001 Published by Elsevier Science Ltd.
PII: S0040-4039(01)01738-5

Table 1. ^{1}H NMR data of salvianolic acid L (**1**) and its two hydrolytic products **3** and **4**

H	1	3	4
1	4.28 d (1.5)	4.26 d (1.0)	4.26 d (1.0)
2	4.45 s	4.52 s	4.50 s
4	7.54 s	7.68 s	7.69 s
5	6.83 br s	6.82 d (8.1)	6.84 d (8.1)
6	6.83 br s	6.75 d (8.0)	6.76 d (8.0)
12	6.58 d (2.0)	6.58 d (2.1)	6.56 d (2.2)
15	6.57 d (8.8)	6.58 d (8.1)	6.57 d (8.1)
16	6.20 dd (8.2, 2.0)	6.49 dd (8.2, 2.0)	6.42 dd (8.2, 2.1)
2′	6.83 br s		6.77 d (2.0)
5′	6.76 d (8.1)		6.72 d (8.0)
6′	6.67 dd (8.1, 2.0)		6.61 dd (8.1, 2.0)
7′a	2.95 dd (14.1, 9.8)		3.01 dd (14.2, 7.7)
7′b	3.08 dd (14.0, 3.9)		3.08 dd (14.2, 5.0)
8′	4.93 dd (9.8, 3.3)		5.11 dd (7.6, 4.9)
2″	6.48 d (1.7)		
5″	6.57 d (8.8)		
6″	6.11 dd (8.1, 2.0)		
7″a	2.67 dd (14.4, 10.1)		
7″b	2.88 dd (14.2, 2.8)		
8″	4.83 dd (10.0, 3.0)		

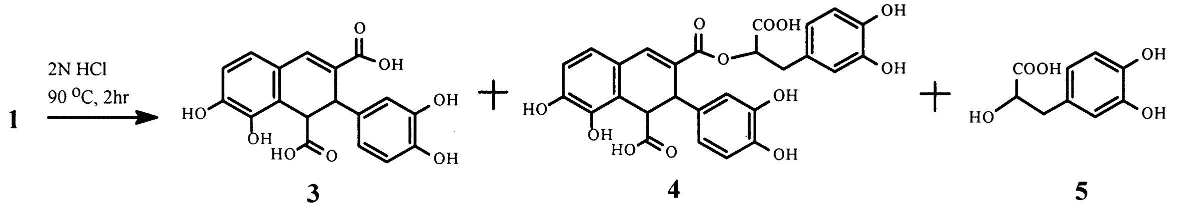
dihydronaphthalene ring structure. This deduction was supported by ES-MS, which showed an [M−H]$^-$ at *m*/*z* 717 consistent with the molecular formula of $C_{36}H_{30}O_{16}$ and being two hydrogen atoms less than the sum of two rosmarinic acid molecules. The remarkable difference in chemical shifts of H-1/H-2 of salvianolic acid L (δ 4.28/4.45) compared to those of rabdosiin (δ 4.56/3.93) or yunnaneic acid G (δ 5.09/4.00) suggested that salvianolic acid L had different substituents on those positions. Further, HMBC experiments showed that the H-2 was long range coupled to C-4 (δ 139.7), C-9 (δ 120.6), C-12 (δ 116.2), C-16 (δ 120.2) and the two carboxylic ester carbons (δ 168.9 and 174.1) while H-1 was correlated with C-3 (δ 127.0), C-8 (δ 144.3), C-10 (δ 125.8) and C-11 (δ 133.7), thus showing that the dihydroxyphenyl group was attached to C-2 of the 1,2-dihydronaphthalene structure. This configuration indicated that the dimerization of rosmarinic acid was head-to-tail in salvianolic acid L in contrast to head-to-head type found in both rabdosiin and yunnaneic acid G. The association between H-1 and C-8 also showed that the two hydroxyl groups in the 1,2-dihydronaphthalene were at C-7 and C-8. Thus, salvianolic acid L was identified as 7,8-dihydroxy-2-(3,4-dihydroxyphenyl)-1,2-dihydronaphthalene-1,3-dicarboxylic acid di(1-carboxy-2-(3,4-dihydroxyphenyl))ethyl ester.

The identity of salvianolic acid L was corroborated by acid hydrolysis (Scheme 1), which yielded two products, 7,8-dihydroxy-2-(3,4-dihydroxyphenyl)-1,2-dihydronaphthalene-1,3-dicarboxylic acid (**3**) and its 3-monoester (**4**) together with the known 3-(3,4-dihydroxyphenyl)lactic acid (**5**). The structures of compounds **3** and **4** were fully elucidated and assigned using 2D NMR techniques.[8] Compound **3** showed similar NMR chemical shifts to those of the relevant moiety of **1**. Evidence for the 7,8-dihydroxy-1,2-dihydronaphthalene ring in the ^{1}H NMR spectrum of **3** was shown by the two mutually coupled doublets at δ 6.82 and 6.75 for H-5 and H-6, respectively. That compound **4** was the monoester of **3** was apparent from both the ^{1}H (Table 1) and ^{13}C NMR data. The position of esterification was deduced to be at the carboxylic acid located on C-3 of the 1,2-dihydronaphthalene ring by HMBC, which showed long range coupling between the quaternary carbon signal at δ 168.4 (C-17) and the doubled doublet proton signal at δ 5.11 (H-8′).

Salvianolic acid L showed potent antioxidant activity as assessed by its capacity to scavenge DPPH and superoxide anion radicals.[4,9] A comparison of its potency with trolox with the activity expressed as EC_{50} (the effective concentration of antioxidant necessary to decrease the initial DPPH by 50%) and the SOD (superoxide dismutase) equivalent activity (units/mg) is shown in Table 2. Salvianolic acid L was a significantly better scavenger of these free radicals than trolox, caffeic acid and rosmarinic acid, the latter being the major phenolic antioxidant in sage.

Table 2. Antioxidant activities of salvianolic acid L (**1**)[10]

Compound	DPPH (EC_{50})	SOD (units/mg)
Salvianolic acid L	0.09	300
Trolox	0.48	15
Caffeic acid	0.24	260
Rosmarinic acid	0.21	230



Scheme 1. Acid hydrolysis of salvianolic acid L (**1**).

Acknowledgements

The authors gratefully thank Dr. Herbert Wong for the provision of NMR data and the NZ FRST foundation (C0 8811-4) for financial support.

References

1. (a) Chipault, J. R.; Mizuno, G. R.; Lundberg, W. O. *Food Technol*. **1956**, *10*, 209–211; (b) Cuvelier, M.-E.; Richard, H.; Berset, C. *J. Am. Oil Chem. Soc*. **1996**, *73*, 645–652.
2. (a) Wang, M.; Li, J.; Rangarajan, M.; Shao, Y.; La Voie, E. J.; Huang, T.-C.; Ho, C.-T. *J. Agric. Food Chem*. **1998**, *46*, 4869–4873; (b) Wang, M.; Shao, Y.; Li, J.; Zhu, N.; Rangarajan, M.; La Voie, E. J.; Ho, C.-T. *J. Nat. Prod*. **1999**, *62*, 454–456.
3. (a) Lu, Y.; Foo, L. Y. *Phytochemistry* **1999**, *51*, 91–94; (b) Lu, Y.; Foo, L. Y.; Wong, H. *Phytochemistry* **1999**, *52*, 1149–1152; (c) Lu, Y.; Foo, L. Y. *Phytochemistry* **2000**, *55*, 263–267.
4. Lu, Y.; Foo, L. Y. *Food Chem*. **2001**, *75*, 197–202.
5. Salvianolic acid L: ^{1}H NMR (300 MHz, D_2O/CD_3OD) see Table 1; ^{13}C NMR (75 MHz, D_2O/CD_3OD) δ 37.95 (C-7″), 38.50 (C-7′), 40.38 (C-2), 47.41 (C-1), 77.92 (C-8′), 78.44 (C-8″), 115.66 (C-6), 116.18 (C-12), 116.90 (C-15), 116.98 (C-5′), 117.09 (C-5″), 117.39 (C-2″), 118.09 (C-2′), 120.17 (C-16), 120.55 (C-9), 122.21 (C-6″), 122.66 (C-6′), 123.73 (C-5), 125.80 (C-10), 127.00 (C-3), 130.83 (C-1″), 131.51 (C-1′), 133.67 (C-11), 139.73 (C-4), 143.80 (C-4″), 144.08 (C-14), 144.31 (C-8, C-4′), 144.99 (C-3″), 145.30 (C-13), 145.40 (C-3′), 149.72 (C-7), 168.88 (C-17), 174.14 (C-18), 177.77 (C-9″), 178.09 (C-9′); ES-MS (negative mode) m/z 717.2 ($[M-H]^-$, 20%), 358.5 ($[M-2H]^{2-}$, 100%); UV–vis λ_{max}^{MeOH} ($\log \varepsilon$) 212 (4.48), 288 (4.05), 334 nm (4.12); $[\alpha]_D$ −177.5 (*c* 0.2, MeOH).
6. (a) Agata, I.; Hatano, T.; Nishibe, S.; Okuda, T. *Phytochemistry* **1989**, *28*, 2447–2450; (b) Nishizawa, M.; Tsuda, M.; Hayashi, K. *Phytochemistry* **1990**, *29*, 2645–2649.
7. Tanaka, T.; Nishimura, A.; Kouno, I.; Nonaka, G.; Yang, C.-R. *Chem. Pharm. Bull*. **1997**, *45*, 1596–1600.
8. 7,8-Dihydroxy-2-(3,4-dihydroxyphenyl)-1,2-dihydronaphthalene-1,3-dicarboxylic acid (**3**) (15%): ^{1}H NMR (300 MHz, CD_3OD) see Table 1; ^{13}C NMR (75 MHz, CD_3OD) δ 42.30 (C-2), 47.84 (C-1), 115.18 (C-6), 115.89 (C-12), 116.64 (C-15), 120.15 (C-16), 121.11 (C-9), 122.61 (C-5), 126.74 (C-10), 128.19 (C-3), 135.39 (C-11), 139.20 (C-4), 145.43 (C-14), 145.58 (C-8), 146.33 (C-13), 149.57 (C-7), 170.99 (C-17), 176.88 (C-18). 7,8-Dihydroxy-2-(3,4-dihydroxyphenyl)-1,2-dihydro-naphthalene-1,3-dicarboxylic acid 3-(1-carboxy-2-(3,4-dihydroxyphenyl))ethyl ester (**4**) (80%): ^{1}H NMR (300 MHz, CD_3OD) see Table 1; ^{13}C NMR (75 MHz, CD_3OD) δ 38.35 (C-7′), 42.29 (C-2), 47.80 (C-1), 75.12 (C-8′), 115.23 (C-6), 115.82 (C-12), 116.68 (C-15), 116.84 (C-5′), 118.13 (C-2′), 120.21 (C-16), 121.19 (C-9), 122.44 (C-6′), 123.00 (C-5), 126.49 (C-10), 127.08 (C-3), 129.66 (C-1′), 135.17 (C-11), 140.01 (C-4), 145.43 (C-14), 145.58 (C-8, C-4′), 146.29 (C-13), 146.49 (C-3′), 149.82 (C-7), 168.43 (C-17), 173.81 (C-9′), 176.77 (C-18).
9. (a) Lu, Y.; Foo, L. Y. *Food Chem*. **2000**, *68*, 81–85; (b) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. *Lebensm. Wiss. Technol*. **1995**, *28*, 25–30; (c) Okamura, H.; Mimura, A.; Yakou, Y.; Niwano, M.; Takahara, Y. *Phytochemistry* **1993**, *33*, 557–561.
10. The antioxidant activities of salvianolic acid L, together with other sage polyphenols, have been orally presented at *The Second International Symposium on Natural Antioxidants: Molecular Mechanisms and Health Effects*. June 4–8, 2001, Beijing, China.