β-Sitosterol Lithospermate from *Salvia Columbariae*

James D Adams Jr*., Steven Tran, Vincent Wong, Pauline Fontaine, and Jirair Petrosyan

Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA, USA

Abstract: *Salvia columbariae* extracts were examined for the presence of salvianolic acids by HPLC-MS and NMR. A new salvianolic acid, β-sitosterol lithospermic acid, was found.

Keywords: *Salvia columbariae*, chia, β-sitosterol lithospermate, salvianolic acid, functional food, stroke, heart attack.

INTRODUCTION

Salvia columbariae Benth. (Lamiaceae) is an annual sage called chia and is found in the American west from California to Utah and south into Mexico [1]. The Chumash Indian name for the plant is 'ilepesh [1]. The plant is grown by Chumash and other California Indians, is used for food and as a treatment for stroke and heart attack [1, 2]. Previous examinations of plant extracts have found cryptotanshinone, miltionone II and tanshinone IIA [2]. These compounds are also found in *Salvia miltiorrhiza* (Lamiaceae), the plant used in China as a treatment for stroke and heart attack [3]. Other diterpenoids identified in the aerial parts of *S. columbariae* include: 11,12-di-O-methylrosmanol, 11,12-di-O-methylcarnosol, carnosic acid, carnosol, rosmanol, epirosmanol, isorosmanol, salvicanol and rosmadial [4]. *S. miltiorrhiza* is known to contain salvianolic acids [3]. Tanshinones have been shown to be neuroprotective and anti-inflammatory [3]. Salvianolic acids are neuroprotective, increase cerebral blood flow and inhibit platelet aggregation [3]. The current paper reports an examination of *S. columbariae* for salvianolic acids that may be of interest in stroke treatment.

MATERIALS AND METHODS

General Experiment Procedures

The ^{1}H NMR spectra were recorded at 400 MHz with TMS as an internal standard. The ^{13}C NMR spectra were performed at 400 MHz. Plant extracts were fractionated by column chromatography on Silica gel 60. Four fractions were collected by eluting with dichloromethane. A fifth fraction was collected by eluting with 20% methanol in dichloromethane. Fractions were examined for purity by TLC on Silica gel 60. HPLC-MS was performed with a ThermoFinnigan LCQ Deca system. The chromatography involved an Eclipse XDB-C18 column (250x4.6mm i.d., Phenomenex) developed with 1 ml/min of 80/20 methanol/water containing 1% formic acid. High resolution HPLC-MS employed a ThermoFinnigan LCQ ion trap operated in electrospray positive ion mode. Fragmentation of most peaks was studied in MS/MS mode. Chromatography was as described for the low resolution system.

Plant Material

S. columbariae was collected in a city park near Pasadena, California, USA with permission from the park administration. Four plants were collected from the 40 plants found. The plants were identified by J. Adams. Plant vouchers of *S. columbariae* can be found at the Rancho Santa Ana Botanic Gardens, Claremont, California, USA.

EXTRACTION

S. columbariae roots were extracted by microwave extraction [2]. A sample of 11.4 g or root material was extracted into 50 ml of 90% ethanol. After filtration, the extract was examined by HPLC-MS and fractionated by silica gel column chromatography.

β-sitosterol lithospermic acid 1: brown solid 4.7 mg; $[\alpha]_D^{20}$ = -17.70; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (m, 6H, H-4, H-11, H-16, H-23, H-29, H-34), 7.52 (s, 1H, H-7), 7.25 (s, 1H, H-6), 7.07 (s, 1H, H-22), 6.88 (m, 5H, H-14, H-18, H-25, H-26, H-32), 6.75-6.77 (m, 4H, H-5, H-17, H-35, H-36), 6.69 (s, 1H, H-8), 6.25 (m, 1H, H-20), 5.36 (m, 4H, H-10, H-6', H-6", H-6"'), 5.18-5.27 (d, *J* = 1 Hz, 1H, H-28), 4.74 (d, *J* = 2 Hz, 1H, H-19), 4.17-4.23 (m, 3H, H-3', H-3", H-3"'), 2.95-3.16 (m, 4H, H-12, H-30), 2.26-2.33 (m, 12H, H-1', H-1", H-1"', H-4', H-4", H-4"'), 1.83-2.07 (m, 27H, H-2', H-2", H-2"', H-7', H-7", H-7"', H-8', H-8", H-8"', H-12', H-12", H-12"', H-15', H-15", H-15"', H-16', H-16", H-16"', H-25', H-25", H-25"', H-28', H-28", H-28"'), 1.43-1.68 (m, 12H, H-2', H-2", H-2"', H-9', H-9", H-9"', H-11', H-11", H-11"'), 1.09-1.35 (m, 45H, H-12', H-12", H-12"', H-14', H-14", H-14"', H-15', H-15", H-15"', H-16', H-16", H-16"', H-17', H-17", H-17"', H-19', H-19", H-19"', H-20', H-20", H-20"', H-22', H-22", H-22"', H-23', H-23", H-23"', H-24', H-24", H-24"', H-28', H-28", H-28"'), 0.81-0.93 (m, 36H, H-21', H-21", H-21"', H-26', H-26", H-26"', H-27', H-27", H-27"', H-29', H-29", H-29"'), 0.65 (m, 9H, H-18', H-18", H-18"'); ^{13}C NMR (400 MHz, $CDCl_3$) δ 173.05 (2C, C-11, C-29), 168.95 (C-27), 164.89 (C-9), 150.44 (4C, C-3, C-16, C-

*Address correspondence to this author at the Department of Pharmacology and Pharmaceutical Sciences,School of Pharmacy, University of Southern California, 1985 Zonal Avenue, PSC 716, Los Angeles, CA 90089-9121, USA; Tel: +001 323 442 1362; Fax: +001 323 442 1681;
E-mail: jadams@pharmacy.usc.edu

1874-8481/12

23, C-34), 146.56 (5C, C-4, C-7, C-15, C-24, C-33), 140.39 (3C, C-5', C-5", C-5'"), 132.60(C-21) , 127.60 (C-31), 126.52 (C-13), 121.73 (4C, C-1, C-2, C-6, C-18), 120.90 (4C, C-6', C-6", C-6'", C-36), 115.87 (5C, C-5, C-8, C-14, C-26, C-32), 115.29 (4C, C-17, C-22, C-25, C-35), 81.46 (C-20), 77.33 (3C, C-3', C-3", C-3'"), 76.69 (2C, C-10, C-28), 55.23 (4C, C-14', C-14", C-14'", C-19), 53.31 (3C, C-17', C-17", C-17'"), 50.11 (3C, C-9', C-9", C-9'"), 45.61 (3C, C-24', C-24", C-24'"), 42.29 (3C, C-13', C-13", C-13'"), 38.17 (3C, C-4', C-4", C-4'"), 37.24 (3C, C-12', C-12", C-12'"), 36.50 (5C, C-10', C-10", C-10'", C-12, C-30), 36.15 (3C, C-1', C-1", C-1'"), 34.58 (3C, C-20', C-20", C-20'"), 34.06 (3C, C-22', C-22", C-22'"), 33.15 (3C, C-8', C-8", C-8'"), 31.65 (3C, C-7', C-7", C-7'"), 29.71 (6C, C-2', C-2", C-2'", C-25', C25", C-25'"), 28.59 (6C, C-15', C-15", C-15'", C-16', C-16", C-16'"), 26.41 (3C, C-23', C-23", C-23'"), 24.86 (3C, C-19', C-19", C-19'"), 23.71 (3C, C-28', C-28", C-28'"), 22.70 (3C, C-11', C-11", C-11'"), 21.08 (3C, C-18', C-18", C-18'"), 19.87 (3C, C-26', C-26", C-26'"), 19.02 (3C, C-27', C-27", C-27'"), 18.84 (3C, C-21', C-21", C-21'"), 11.98 (3C, C-29', C-29", C-29'"); ESI MS 689 $[C_{35}H_{29}O_{15}]^+$, 527 $[504+Na]^+$, 504 $[C_{26}H_{16}O_{11}]^+$, 399 $[C_{29}H_{51}]^+$, 381 $[342+K]^+$, 365 $[342+K]^+$, 342 $[C_{17}H_{10}O_8]^+$; HRMS (ESI) fragment calcd for $C_{86}H_{115}O_{13}$ 1355.8338, found 1355.5435, lithospermate fragment calcd for $C_{35}H_{29}O_{15}$ 689.1507, found 689.1995. X-Ray crystallography of crystals of **1** grown in dichloromethane produced inconclusive results.

RESULTS

Initial low resolution HPLC-MS of *S. columbariae* extracts demonstrated a peak that eluted at 0.55 min and was the major peak on the total ion current chromatogram. The peak was comprised of a compound similar to salvianolic acid B, here named β-sitosterol lithospermic acid, **1** (Fig. **1**). The compound was characterized by mass fragmentation and was found to lose β-sitosterol resulting in fragments of lithospermate (MW 718, $C_{36}H_{30}O_{16}$) [3]. β-Sitosterol was also found as a fragment (MW 399, $C_{29}H_{51}$). However, the molecular ion for the parent compound (1908.6881, $C_{123}H_{174}O_{16}$) was not found since the mass spectrometer could not be calibrated for masses above 1200. A fragment was found at 1355.5435 that corresponds to loss of a β-sitosterol and part of the lithospermate (M-$C_{37}H_{59}O_3$). However, the calibration was not reliable for this fragment.

^{1}H NMR and ^{13}C NMR found signals for lithospermate and β-sitosterol. An HMBC NMR experiment of **1** showed that the sterols connect with lithospermate: 3' connects with

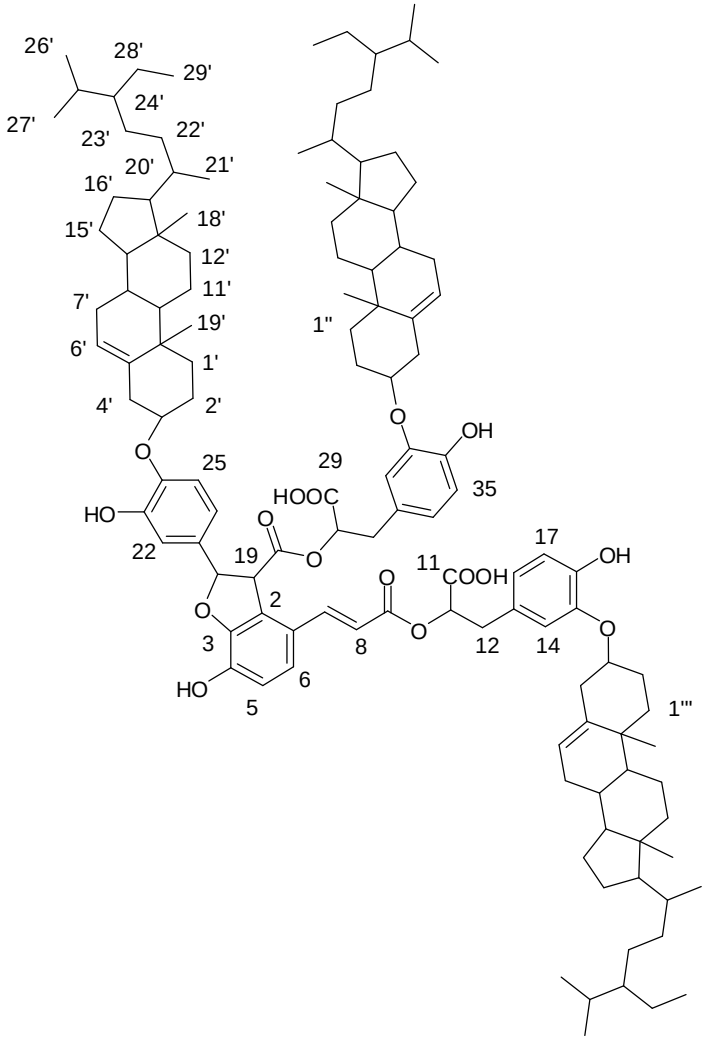

Fig. (1). The chemical structure of **1** with numbering.

23, 3" connects with 34 and 3" connects with 16. Connections were found between lithospermate carbons and protons: 19 connects with 28 and 30. There were numerous connections between the sterol carbons and the sterol side chains, such as 20' connects with 15' and 16', 20" connects with 15" and 16", 20"' connects with 15"' and 16"'. A TOCSY NMR experiment found numerous sterol proton connections such as 1' connects with 12' and 14', 4' connects with 9' and 11', 11' connects with 17'. The structural assignment was confirmed by the ACD Labs NMR predictor program. X-Ray crystallography of the compound was inconclusive implying that the crystals were not of high enough quality.

DISCUSSION

The mass spectrometry of β-sitosterol lithospermic acid demonstrated that it had a base peak ion corresponding to $C_{35}H_{28}O_{15}$. Several fragments were found corresponding to molecular weights of 168 (loss of $C_8H_8O_4$), 181 (loss of $C_9H_9O_4$) and 185 (loss of $C_8H_9O_5$). ^{1}H NMR was marked by signals for β-sitosterol and lithospermate. The signals were shown to connect by HMBC and TOCSY experiments. The absolute configuration of lithospermic acid B has been assigned [5].

A cholesterol ester of a phospholipid was found in the *S. columbariae* extract. The general structure of this compound was verified by ^{1}H NMR [6].

ACKNOWLEDGEMENTS

The authors thank Dr. Mona Shahgholi of Caltech for helping with the HPLC-MS work.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

REFERENCES

[1] Garcia, C.; Adams, J.D. *Healing with medicinal plants of the west – cultural and scientific basis for their use.* 2nd ed.; Abedus Press: La Crescenta, **2009**; pp 182-184.

[2] Adams, J.D.; Wall, M.; Garcia, C. Salvia columbariae contains tanshinones. *Evid. Based Complement Alternat. Med.,* **2005**, *2*, 107-110.

[3] Adams, J.D.; Wang, R.; Yang, J.; Lien, E. Preclinical and clinical examinations of Salvia miltiorrhiza and its tanshinones in ischemic conditions. *Chin. Med.*, **2006**, *1*, 14-28.

[4] Luis, J.G.; Quinones, W.; Grillo, T.A.; Kishi, M.P. Diterpenes from the aerial part of Salvia columbariae. *Phytochemistry*, **1994**, *35*, 1373-1374.

[5] Watzke, A.; O'Malley, S.J.; Bergman, R.G.; Ellman, J.A. Reassignment of the configuration of salvianolic acid B and establishment of its identity with lithospermic acid B. *J. Nat. Prod.,* **2006**, *69,* 1231-1233.

[6] Subramanian, A.; Joshi, B.S.; Roy, A.D.; Roy, R.; Gupta, V.; Dang, R.S. NMR spectroscopic identification of cholesterol esters, plasmalogen andphenolic glycolipids as fingerprint markers of human intracranial tuberculomas. *NMR Biomed*., **2008**, *21*, 272-288.

Received: May 08, 2012 Revised: June 24, 2012 Accepted: June 26, 2012