

Tryptophan and Sterols from *Salvia limbata*

Saeidnia S (Ph.D.)¹, Gohari AR (Ph.D.)^{1*}, Malmir M (B.Sc.)¹, Moradi-Afrapoli F (Pharm.D.)¹,
Ajani Y (M.Sc.)²

1- Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

2- Department of Pharmacognosy, Institute of Medicinal Plants, ACECR, Tehran, Iran

*Corresponding author: Medicinal Plants Research Center, Tehran University of Medical Sciences, P.O.Box: 14155 - 6451, Tehran, Iran

Tel & Fax: +98 – 21 - 64122330

Email: goharii_a@tums.ac.ir

Receive: 26 Dec. 2010

Acceptance: 5 Feb. 2011

Abstract

Background: *Salvia limbata* is an aromatic herbaceous plant and grows widely in Iran Turkey and Afghanistan. In the previous study, six flavones together with rosmarinic acid were isolated from the ethyl acetate and methanol extracts of *S. limbata*. In this report, we focused on the isolation and identification of the glycosylated sterols and the main accumulated amino acid of the species *S. limbata*, which has not been previously reported.

Methods: Aerial parts of the plant were dried, cut into small pieces and extracted with ethyl acetate and methanol by percolation at room temperature. The separation process was carried out using several chromatographic methods. Structural elucidation was based on NMR data, in comparison with those reported in the literature.

Results: The isolated compounds (Figure 1) from the ethyl acetate and MeOH extracts of *S. limbata* were identified as beta-sitosterol (1), stigmasterol (2), daucosterol (3), stigmasterol 3-O-glucoside (4) and tryptophan (5) by comparison of their NMR spectral data with those reported in the literature

Conclusions: *S. limbata* can accumulate the tryptophan as a major free amino acid together with sterols and their glucosides. Therefore, consumption of *S. limbata* (as a herbal tea or other preparations), which contains the essential amino acid tryptophan, might be useful for dietary deficiency of tryptophan.

Keywords: *Salvia limbata*, Daucosterol, Stigmasterol- 3-O-glucoside, Tryptophan

Introduction

The genus *Salvia* (*Lamiaceae*) includes nearly 900 species spread throughout the world. This genus is represented in Iran by 58 species [1, 2]. Many *Salvia* species are used as herbal tea and flavouring agent as well as in cosmetics, perfumery and the pharmaceutical industries [2]. *Salvia* species have been used in folk and traditional medicines as tonic, anti-rheumatoid, antimicrobial, carminative and flavored spice since antiquity [3].

As many as 107 species of *Salvia* has been recognized in the *Flora Orientalis*. These species have been placed under seven sections using Bentham's (1833) sectional delimitation. After that, the section *Eusphace* was changed to the section *Salvia* [4, 5]. *Salvia limbata* C.A. Mey., was placed in the section *Aethiopis*. The section *Aethiopis* comprises biennial or perennial herbs. The characteristic features of the section are tubular or campanulate calyx, more or less falcate upper lip of corolla and not annulate corolla tube. Staminal connectives are longer than filaments, arms are unequal, the sterile shorter and more or less flattened distally [5].

Salvia limbata is an aromatic herbaceous plant and grows widely in Iran Turkey and Afghanistan [1, 6]. It was well known that the study of the phytochemicals in medicinal plants, such as *Salvia*, could be useful for indication of chemical diversity, chemotaxonomy as well as pharmacological and biological activities [7]. In our previous investigation, six flavones (ladanein, salvigenin, luteolin 7-methyl ether, cirsiolol, eupatorin and luteolin 7-O-glucoside) together with rosmarinic acid were isolated from the ethyl acetate and methanol extracts of *S. limbata* [8]. In this paper, we focused on the isolation and identification of the glycosylated sterols and the main accumulated amino acid

of the species *S. limbata*, which has not been previously reported.

Experimental

Plant materials

Aerial part of *S. limbata*, at the full flowering stage, was collected from the Ahovan area in Semnan Province (July, 2008). A voucher specimen of the plant was deposited at the Herbarium of the Institute of Medicinal Plants, ACECR, Tehran.

Extractions of the plants

Aerial parts of the plants were dried at room temperature and reduced to small pieces, followed by extraction with ethyl acetate and methanol using percolation. Then the solvents evaporated under reduced pressure to obtain the concentrated extracts and dried under freeze dryer to obtain powder of the extracts.

Instruments

^1H and ^{13}C -NMR spectra were measured on a Bruker Avance TM 500 DRX (500MHz for ^1H and 125MHz for ^{13}C) spectrometer with tetramethylsilane as an internal standard; chemical shifts are given in δ (ppm). Pre-coated silica gel 60F₂₅₄ plates (Merck TM) were used for TLC. Spots were detected by spraying anisaldehyde- H_2SO_4 reagent followed by heating.

Isolation

The dried aerial parts of *S. limbata* (836 g) were cut into small pieces and extracted with ethyl acetate and methanol, respectively, at room temperature. The ethyl acetate extract (38.4 g) was subjected to silica gel column chromatography (CC) with hexane: AcOEt (19: 1, 9: 1, 0: 1) and methanol to give four fractions (A–D). Fraction C (238 mg) was submitted to silica gel CC with hexane: AcOEt



(8: 2), yielding seven fractions (C₁–C₇). Fraction C₅ was the mixture of compounds **1** and **2** (g).

The MeOH extract (20 g) was successively subjected to silica gel column chromatography with CHCl₃: MeOH (8: 2, 6: 4, 4: 6 and 0: 1) to result in five main fractions, M₁–M₅. Fraction M₃ (61 mg) was submitted to silica gel CC with CHCl₃: MeOH (9: 1) to obtain six fractions, M₃₁–M₃₆. The fraction M₃₄ (30 mg) was submitted to sephadex LH₂₀ with MeOH to yield mixed compounds **3** and **4** (11 mg).

Fraction M₅ (2.4 g) was fractionated on the reverse phase (C18) silica gel CC with MeOH: H₂O (3: 7, 1: 1, 7: 3, 0:1) to result in eight fractions (M₅₁–M₅₈). The fraction M₅₂ (532 mg) was purified on sephadex LH₂₀ twice with MeOH to yield compound **5** (19 mg).

Results

The isolated compounds (Figure 1) from the ethyl acetate and MeOH extracts of *S. limbata* were identified as beta-sitosterol (**1**), stigmasterol (**2**), daucosterol (**3**), stigmasterol- 3- O-glucoside (**4**) and tryptophan (**5**) by comparison of their ¹H and ¹³C -NMR spectral data with those reported in the literature [9-11]. The ¹³C -NMR data of the isolated sterols are summarized in Table 1. Also, the ¹H-NMR, ¹³C -NMR, HMBC, HSQC and ¹H-¹H COSY data of the isolated amino acid are indicated in Table 2. The main and important HMBC correlations of the compound **5** are shown in Figure 2, in

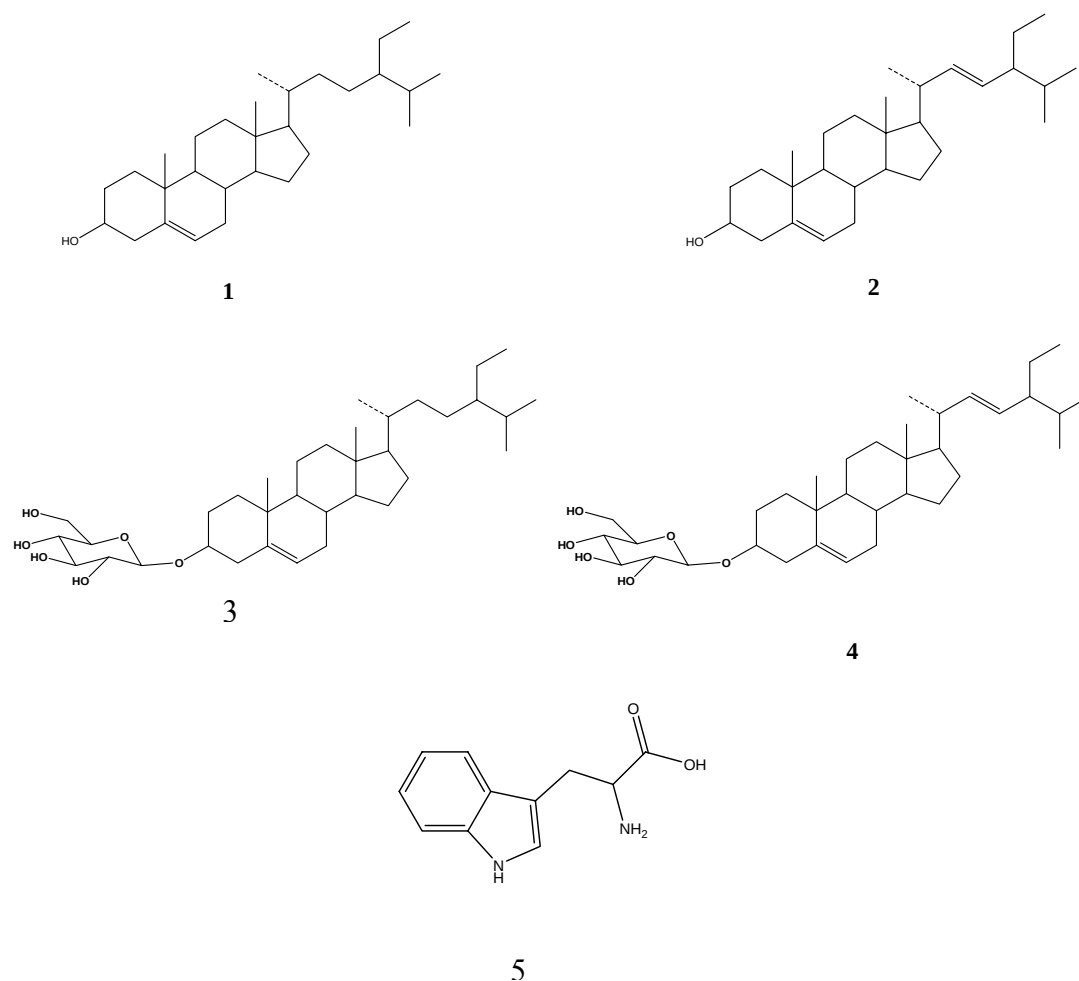


Figure 1- Chemical structures of the isolated compounds from the *S. limbata*

Table 1- The ^{13}C –NMR data of the isolated sterols from *S. limbata*

Carbon No	1	2	3	4
1	37.3	37.3	37.4	37.4
2	31.7	31.7	30.2	30.2
3	71.8	71.8	78.5	78.5
4	42.3	42.2	39.2	43.9
5	140.8	140.8	140.9	141.5
6	121.7	121.7	121.9	121.1
7	31.9	31.9	32.0	31.9
8	31.9	31.9	31.9	31.9
9	50.2	50.2	50.2	50.7
10	36.4	36.4	36.8	36.9
11	21.1	21.1	21.1	21.1
12	39.8	39.7	39.8	39.9
13	42.3	42.2	42.4	43.0
14	56.8	56.9	56.7	56.9
15	24.3	24.4	24.4	24.5
16	28.3	28.9	28.4	28.9
17	56.1	56.0	56.1	56.0
18	11.9	12.0	11.8	12.0
19	19.8	19.4	19.3	19.4
20	36.2	40.5	36.3	40.5
21	18.8	21.2	18.9	21.9
22	34.0	138.3	34.1	138.9
23	26.1	129.3	26.2	129.1
24	45.8	51.6	45.9	52.1
25	29.2	31.9	29.3	32.9
26	19.0	19.0	19.1	19.0
27	19.4	21.1	19.8	21.7
28	23.1	25.4	23.2	25.6
29	12.0	12.2	12.0	12.2
Glc-1	-	-	102.5	102.8
Glc-2	-	-	75.3	74.2
Glc-3	-	-	78.6	79.8
Glc-4	-	-	71.6	70.6
Glc-5	-	-	78.1	76.7
Glc-6	-	-	62.5	62.3

order to appear how 2D-NMR data can be useful for elucidation of the structure of tryptophan.

Discussion

The amino acid, tryptophan, showed a deep and dark purple spot on thin layer chromatography (silica gel 60F₂₅₄ plates) by

spraying anisaldehyde-H₂SO₄ reagent followed by heating (120° C, 5 minutes). The isolated sterols showed the same spots (from purple to dark blue) as compound 5. This paper is the first report of the sterol glucosides and free tryptophan from *S. limbata*.



Table 2- ^1H - NMR, ^{13}C - NMR, HSQC and HMBC data of the isolated amino acid from the *S. limbata*

Carbon No	HSQC (δ_C)	HSQC (δ_H)	HMBC	1H - 1H COSY
1(NH)	-	10.8	-	H-2
2	124.3	7.19 (1H, <i>d</i> , <i>J</i> =1.5 Hz)	H-8a	H-3, H-1
3	108.6	-	H-2, H-8a, H-4, H-9	-
4	118.3	7.57 (1H, <i>d</i> , <i>J</i> = 8 Hz)	H-6	-
5	118.4	6.98 (1H, <i>t</i> , <i>J</i> = 7Hz)	-	-
6	121.0	7.06 (1H, , <i>t</i> , <i>J</i> = 7Hz)	H-4	-
7	111.3	7.33 (1H, <i>d</i> , <i>J</i> = 8.5 Hz)	H-5	-
3a	127.1	-	H-5, H-8a	-
7a	136.3	-	H-4, H-6	-
8	26.8	2.94 (1Ha, <i>dd</i> , <i>J</i> = 1.5Hz)	-	H-9
		3.10 (1Hb, <i>brs</i>)		
9	54.0	3.50 (1H, <i>m</i>)	-	H-8a
10	170.3	-	H-8a, H-9	-

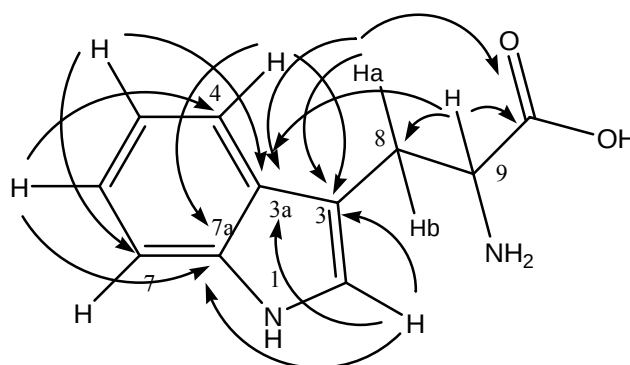


Figure 2. HMBC correlations of the amino acid tryptophan.

There are several papers about the isolation of sterol glycosides from various plant materials such as sweet potato, soy beans, cotton seed, peanuts, rapeseed and linseed but, there are only few reports on the biological activity of those glycosides in the inhibition of the growth of *Fusarium lini* [12]. It has been reported that the synthetic beta-sitosterol- D-glucoside (daucosterol) showed growth-promoting properties approximately equal to natural one and more active than free beta-sitosterol [12]. The presence of an auxin

synergist was confirmed in the neutral fraction of *Arachis hypogaea*. Stigmasteryl-beta-D-glucoside was responsible for the activity as it promoted markedly the elongation of *Avena* coleoptile segments induced by indole acetic acid [13]. Beta-sitosterol is present in all plant lipids and is used for steroid synthesis. Stigmasterol is used for the production of progesterone and vitamin D₃ and is a potential anti-inflammatory compound. It is found that its activity might be mediated by the inhibition of several pro-inflammatory and matrix degradation mediators

involved in osteoarthritis-induced cartilage degradation [14].

Tryptophan is one of the ten essential amino acids that the human body uses to synthesize the proteins it needs. Its role in the production of nervous system messengers is well-known. A small amount of the tryptophan is converted into niacin (vitamin B3) by the liver. This conversion can help prevent the symptoms associated with niacin deficiency when dietary intake of this vitamin is low. Tryptophan is a precursor for serotonin, a neurotransmitter that helps the body regulate appetite, sleep patterns, and mood. Therefore, it has been used therapeutically in the treatment of insomnia, depression and anxiety [15, 16]. Among the herbs, asparagus, broccoli, mustard seeds, cauliflower and turnip greens are the natural sources of tryptophan in the human diet [17]. In conclusion, the results of this study show

that *S. limbata* can accumulate the tryptophan as a major free amino acid together with sterols and their glucosides. Therefore, consumption of *S. limbata* (as a herbal tea or other preparations), which contains the essential amino acid tryptophan, might be useful for dietary deficiency of tryptophan. Its deficiency may cause the characteristic symptoms of protein deficiency, including weight loss and impaired growth in infants and children. Nowadays, tryptophan has been given therapeutically, as a prescription medicine or dietary supplement, in doses exceeding five grams per day but its side effects of high dosage consumption must be regarded.

Acknowledgements

This research is supported by a Tehran University of Medical Sciences and Health Services grant (No. 8177).

References

1. Hedge IC. Labiatae In: Rechinger KH (ed.), *Flora Iranica, Labiatae*, Akademische Druck-u Verlagsanstalt. Austria. 1986, pp: 403 – 80.
2. Chalchat JC, Michet A, Pasquier B. Study of clones of *Salvia officinalis* L. yields and chemical composition of essential oil. *Flav. Frag. J.* 1998; 13: 68 - 70.
3. Yinrong LL and Foo Y. Polyphenolics of *Salvia*, a review. *Phytochem.* 2002; 59: 117 - 40.
4. Hedge IC. Flora Europaea In: Tutin TG, Heywood VH, Burgess NA, Moore DM, Valentine DH, Walters SM, Webb DA (eds.), *Salvia* L. Cambridge University Press. Cambridge. 1972, pp: 188 - 92.
5. Kahraman A, Dogan M. Comparative study of *Salvia limbata* C. A. and *S. palaestina* Benth (sect. Aethiopis Benth, Labiatae) from East Anatolia, Turkey. *Acta Bot. Croat.* 2010; 69 (1): 47 - 64.
6. Guner A, Ozhatay N, Ekim T, Baser KHC. Flora of Turkey and the East Aegean Islands (11). Edinburgh University Press. Edinburgh. 2000.
7. Tosun M, Ercisli S, Sengul M, Ozer H, Polat T and Ozturk E. Antioxidant properties and total phenolic content of eight *Salvia* species from Turkey. *Biol. Res.* 2009; 42: 175 - 81.
8. Gohari AR, Saeidnia S, Malmir M, Hadjiakhoondi A and Ajani Y. Flavones and rosmarinic acid from *Salvia limbata*. *Nat. Prod. Res.* 2010; 24 (20): 1902 - 6.
9. Gohari AR, Saeidnia S, Hadjiakhoondi A and Honda G. Isolation and identification of four sterols from Oud. *J. Med. Plants* 2008;



28: 47 - 55.

10. Goad LJ, Akihisa T. Analysis of Sterols. Blackie London Academic and Professional. London. 1997, pp: 375 - 6.

11. Saeidnia S, Moradi Afrapoli F, Gohari AR, Malmir M. Cytotoxic flavonoids from *Achillea talagonica* Boiss. *J. Med. Plants* 2009; 8 (5): 52 - 6.

12. Dewey VC, Kidder GW. Biological activity of sterol glycosides. *Biochem. Pharm.* 1962; 2: 53 - 6.

13. Kimura Y, Tietz A and Tamura S. Stigmasteryl- β -D-glucoside as an auxin synergist. *Planta* 1975; 126 (3): 289 - 92.

14. Gabay O, Sanchez C, Salvat C, Chevy F, Breton M, Nourissat G, Wolf C, Jacques C,

Berenbaum F. Stigmasterol: a phytosterol with potential anti-osteoarthritic properties. *Osteoarthr. Cartil.* 2010; 18 (1): 106 - 16.

15. Bell C, Abrams J, Nutt D. Tryptophan depletion and its implications for psychiatry. *Br. J. Psychiatry* 2001; 178: 399 - 405.

16. Martinez A, Knappskog PM, Haavik J. A structural approach into human tryptophan hydroxylase and its implications for the regulation of serotonin biosynthesis. *Curr. Med. Chem.* 2001; 8 (9): 1077 - 91.

17. The U.S. Food and Drug Administration's "Reference Values for Nutrition Labeling". <http://www.whfoods.com/genpage.php?tname=fag&dbid=22>.