Iranian Journal of Pharmaceutical Research (2003) 39-42
Received: June 2002
Accepted: February 2003

Original Article

Volatile Constituents from the Aerial Parts of *Verbena Officinalis* L. (Vervain)

Mohammadreza Shams Ardakani [a]*, Mahmoud Mosaddegh[b], Alireza Shafaati[c]

[a]*Department of Pharmacognosy, School of Pharmacy, Tehran University of Medical Sciences,* [b]*Traditional Medicine and Materia Medica Research Center, shaheed Beheshti University of Medical Sciences,* [c]*Department of pharmaceutical Chemistry, School of Pharmacy, Shaheed Beheshti University of Medical Sciences.*

Abstract

The volatile constituents of the aerial parts of vervain (*Verbena officinalis* L.) cultivated locally were investigated. Analysis of the volatile fraction by GC and GC-MS is described. Compounds were identified by various methods, the major compounds being 3-hexen-1-ol (7.28%), 1-octen-3-ol (32.76%), linalool (4.66%), verbenone (20.49%) and geranial (7.22%).

Keywords: Verbena officinalis; Verbenaceae; Volatile Oil; Gas Chromatography.

Introduction

Verbena officinalis (Fam. Verbenaceae) is a perennial herb with several stiffly erect stems (30-60 cm) which can be found in West Asia, north Africa and throughout Europe (1). The species has been introduced to many other parts of the world (2) and is commonly known as vervain. The plant has been extensively used. It is known for its antidepressant and anticonvoulsant effect as well as its use for the treatment of jaundice, cough, cold and digestive problem (3). Mabey (4) has also recorded its use for healing liver and gallbladder disease and nervous exhaustion.

This paper describes the steam-distilled volatile fraction of the plant which should not be confused with commercial "Verbena oil" that is obtained from *Lippia citriodora* (5).

Experimental

Plant material and isolation

Plants were cultivated locally from seed and whole plants of *Verbena officinalis that* were collected whilst in flowers and identified by comparison with the literature (6, 7). Fresh material (500 g) was chopped, macerated and subjected to steam distillation in a clevenger type apparatus for 3h and the viscose distillate (0.5 ml) was extracted into dichloromethane, dried over anhydrous sodium sulphate and stored in a sealed container at 5°C prior to analysis.

Analysis

Preliminary analysis of the extract was carried out on a Perkin Elmer 8320B Gas Chromatograph equipped with a capillary column 25 meter × 0.33 mm I.D. BP5 (SGE, Australia). The Instrument was temperature programmed from 50°C to 275°C at 3°C/min (75 min total run). Injector temperature was 250°C and the FID detector was maintained at 280°C. The carrier gas was hellium at a flow rate of 32 ml/min, injection spliter sets at 1:100. The GC Mass spectral data were obtained by a Hewlett Packard GC 5890, coupled to Hewlett Packard mass spectrometry 5972, using ionisation energy 70 eV. The chromatographic conditions for GC-MS were similar to the separation conditions of GC, except for the temparature ramp rate which in this case was 8°C/min.

* Correspoding author:
E-mail: shams.ardakani@itmrc.org

Table 1. Steam distillate of *V. officinalis* on BP-5 column (major peaks).

Peak No.	t_R (min)	Kovat's RI	Relative t_R (t_{RR})	constituent	Identity method
1	3.41	866	0.12	3-Hexen-1-ol	a,b,c.d.e
2	6.53	974	0.23	1-Octen-3-ol	a,b,c,d,e
3	11.02	1102	0.39	Linalool	a,b,c,d,e
4	15.74	1207	0.55	Verbenone	a,b,c,d,e
5	17.92	1270	0.62	Geranial	a,b,c,d,e
6	45.52	1955	1.67	Hexadecanoic acid	a,b,c,d,e,f
7	50.16	2082	1.77	Linolenic acid methyl ester	a,b,c,d,e,f
8	51.10	2107	1.80	-	-

a: Retention Time, b: Kovat's Rertention Index, c: Peak enrichment on co-injection with authentic standard, d: Relative Retention time of sample and standard to internal standard (Pentadecane), e: Mass Spectrum, f: Methylation.

Table 2. Mass spectral data of components identified in the steam distillate of *V. officinalis*.

Peak No.	Identified Compound	Base peak	Major Peaks (abundance, %)
1	3-Hexen-1-ol	41	43(16), 55(33), 67 (78), 82(13), 100(1)
2	1-Octen-3-ol	57	41(22), 43(24), 67(4), 72(13), 85(6), 121(<1, M^+-1)
3	Linalool	71	41(69),43(97),55(60), 69(29),80(28),93(63),154(<1, M^+)
4	Verbenone	107	41 (56), 79(55), 80(49), 91(76), 95(17), 122(13),135(69)
5	Geranial	41	55(9), 53(13), 67(9), 69(61), 123(4), 137(6), 152(4, M^+)
6	Hexadecanoic acid	43	41(93), 60(85), 73(91), 129(32), 185(11), 236(<1, M^+-17), 256(13, M^+)
7	Linolenic acid, methyl ester	79	41(89), 67(76), 95(44), 121(11), 135(10), 150(7), 222(7)

Results and Discussion

The volatile oil obtained by steam distillation from aerial parts of *Verbena officinalis* was low yield of 0.1%. The oil was extracted from distillation apparatus using solvent. The preliminary GC analyses showed the presence of 8 components (Figure 1), of which 7 components identified (Table 1). The individual components were identified using the GC-MS (Table 2). The resulted data were matched by the GC-MS computer library. Identification of the components were further verified by comparison of their retention time with those of authentic samples, measuring Kovat's indices, peak enrichment on co-injection with authentic samples and the relative retention time of the components and authentic samples to internal standard (pentadecane was used as an internal standard). The final

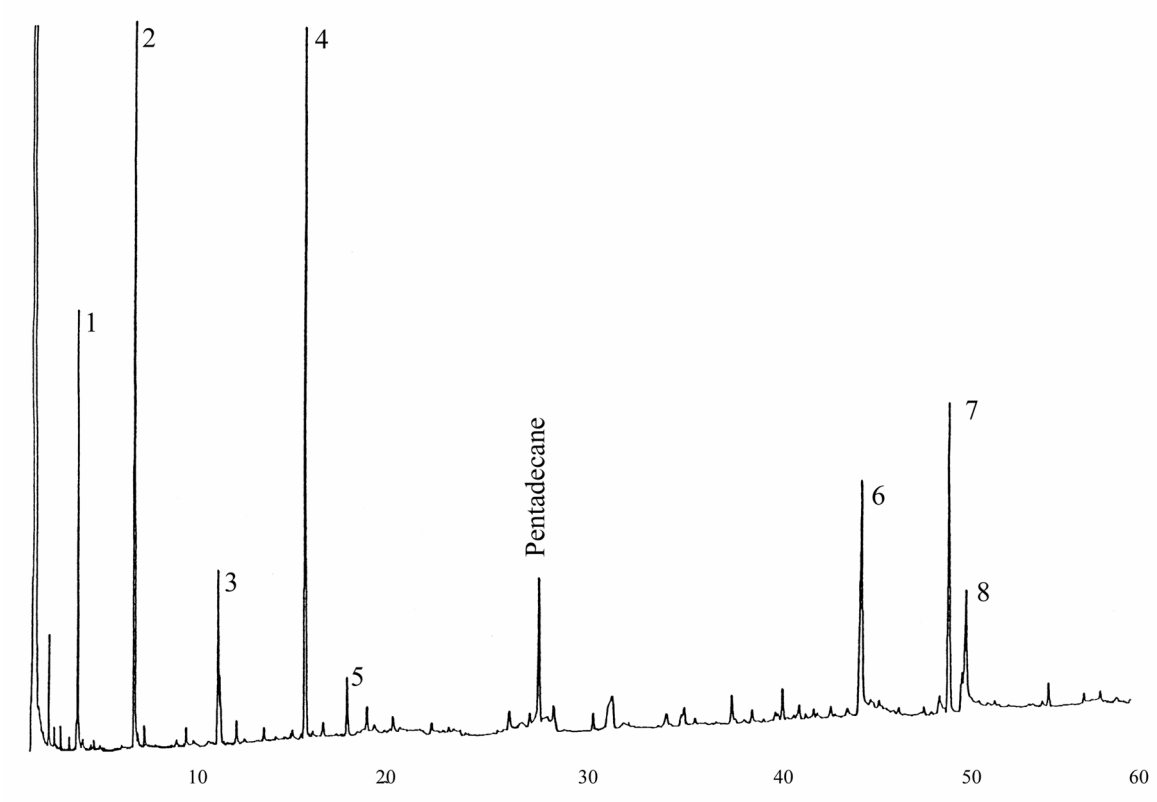

Figure 1. Chromatogram of vervain oil on BP-5 column with added pentadecane.

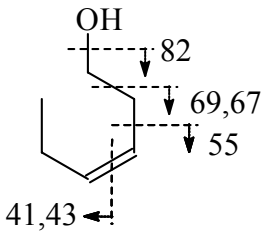

Figure 2. Fragmentation pattern of 3-hexen-1-ol, presented in volatile oil of *V. officinalis.*

identification was based on close study of the fragmentation pattern of each component and comparison of MS data of each component with those reported in the literature (8, 9, 10). The MS data were in agreement with those reported in the literature.

Peak No. 6 has been identified as hexadecanoic acid. To confirm the identification, a part of oil was run on GC and GC-Mass after TMAH derivatization (11). After derivatization the presence of methyl ester of the following compounds was also detected, tetradecanoic acid, hexadecanoic acid, 9-12-octadecadienoic acid, 9-12-15 octadecatrienoic, octadecanoic acid.

Suggested fragmentation profile of some components identified by GC-MS are presented as follows:

1) **3-Hexen-1-ol**- The fragmentation pattern of this hydrocarbone is shown in figure 1. The base peak at m/e 41 and related peak at m/e 43 resulted from cleavage of double bond at C3. The peak at m/e 82 (M^+-18) was due to loss of water which is typical for alcohols. Also, peaks at m/e 55, 67, and 69 all resulted from the fragmentation at α and β carbon of the double bond.

2) **verbenone**- Amongst compounds identified in the steam-distillate of Verbena officinalis was verbenone. Apart from the parent peak (m/e 150), two peaks at m/e 135 and 122 are resulted directly from the parent peak by loss of methyl radical and CO, respectively. Further fragmentation of these two peaks results in the formation of the base peak (m/e 107). The loss of CO is a characteristic feature of ketones. The fragmentation pattern of the compound is given in Figure 3.

3) **Hexadecanoic acid**- One of the unesterified fatty acids identified in the steam distillate of *Verbena officinalis* was

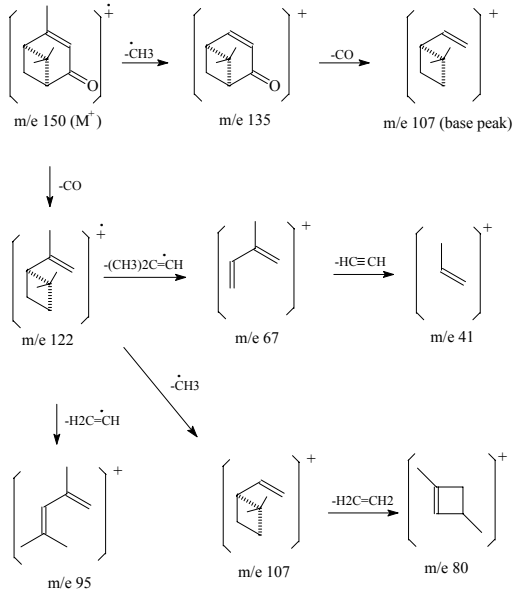

Figure 3. Fragmentation pattern of verbenone identified in the essential oil of *V. officinalis.*

hexadecanoic acid (peak No. 6). The fragmentation pattern of hexadecanoic acid is presented in figure 4. The parent peak at m/e 256 and peaks at m/e 73 and 129 clearly indicate the presence of a carboxylic group attached to a long aliphatic chain. However, the most important peak in the mass spectrum of an aliphatic acid is the peak at m/e 60, which results from a γ-hydrogen shift (11). This hydrogen rearrangement, which is depicted in figure 5 is a characteristic of long chain aliphatic acids and esters (for a methyl ester, the relevant peak would be at m/e 74). Other peaks, including the base peak at m/e 43 resulted from the fragmentation of the aliphatic chain.

In a literature survey, no original reports on the composition of the essential oil of *Verbena officinalis* were found. However, in some textbooks (12, 13, 14, 15) the presence of citral, geraniol, limonene, terpenes, terpene alcohols and verbenone, has been mentioned without addressing the original work. The contents of the steam distillate reported in this work were different from those mentioned above.

Considering that the findings written in the above mentioned texts were based on original observations, the difference between the results of this work and others can be explained on the basis that:

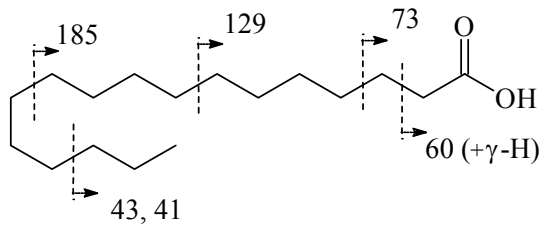

Figure 4. Fragmentation pattern of hexadecanoic acid identified in the steam distillate of *V. officinalis*.

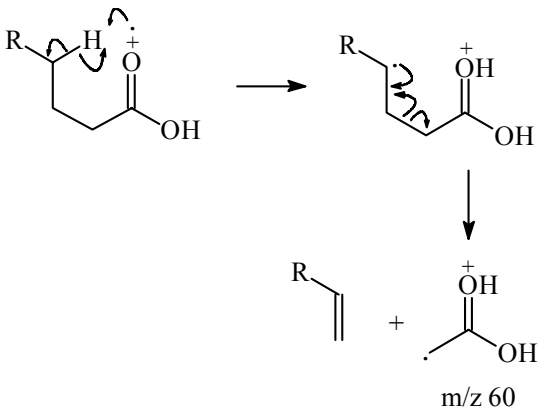

Figure 5. Mechanism of γ-H shift resulted in characteristic peak at m/e 60 which is a typical fragment for long chain aliphatic acids[11].

I) There is a possibility that the above mentioned studies were conducted on the commercial product Verbena oil (which is obtained from a different plant, *Lippia citt.*). The composition of Verbena oil reported by Masada (5) is similar to those quoted in the above texts.

II) Plants are able to produce different secondary metabolites qualitatively and quantitatively in different geographical areas. Also production of crude drugs is subjected to the variation of the climate, to crop disease, to varying methods of collection and drying which influence quality, and to the inherent variation of active constituents arising from plants of the same species, but having different genetic characteristics. Thus, it may be suggested that other researchers work on *V. officinalis* collected in different areas.

References

(1) Clapham AR, Tutin TG and Moore DM. *Flora of the British Isles.* 3rd Ed. Cambridge University Press,Cambridge (1987) 402

(2) Dorfler HP and Roselt G. *The Dictionary of Healing Plants*, Blandford Press (1989) 308

(3) Wren RC. *Potter's New Enyclopaedia of Botanical Drugs and Preparation*, Saffron Walden The C.W. Daniel Company Limited (1988) 275

(4) Mabey R. *The Complete New Herbal*, Elm tree books, London (1988) 128

(5) Masada Y. *Analysis of Essential Oils by Gas Chromatography and Mass Spectroscopy*, John Wiley And Sons, New York (1976)

(6) British Herbal Pharmacopoeia, Part One, British, Herbal Medicine Association,Landon (1980) 221

(7) Hutchinson J. *British Wild Flowers,* Vol.1, David & Charles Ltd,Landon, (1972) 213

(8) Stenhagen E, Abrahamsson S and McLafferty FW. *Atlas of Mass Spectral Data,*John Wiley and sons, New york (1970)

(9) Adams RP. *Identification of Essential Oils by Ion Trap Mass Spectroscopy*, Academic press Inc, Illinois (1989)

(10) McLafferty FW and Turecek F. *Interpretation of Mass Spectra*, 4th Edn. University Science Books, Mill Valley, California (1993)

(11) Blau K and King G (eds.) *Handbook of Derivatives for Chromatography*, Heyden and Son, London (1993)

(12) Duke JA. *CRC Handbook of Medicinal Herbs*, CRC Press, Florida (1985)

(13) Keys JD. *Chinese Herbs, Their Botany, Chemistry and Pharmacodynamic*, Charles E. Tuttle Company (1976)

(14) Palma L. *Le Piante Medicinali d'Italia,* Societa Editice Internazionale, Rome (1964)

(15) Roi SJ. *Traile des Plantes Medicinales Chinoises*, Lechevalier, Paris (1955)