ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 5:3 (2011) 221-227

records of natural products

Determination of Characteristic Components in Essential Oils from *Wisteria brachybotrys* Using Gas Chromatography-Olfactometry Incremental Dilution Technique

Mitsuo Miyazawa*, Shinsuke Marumoto, Tomohiro Kobayashi,

Shuhei Yoshida and Yuya Utsumi

Department of Applied Chemistry, Faculty of Science and Engineering, Kinki University,

3-4-1, Kowakae, Higashiosaka-shi, Osaka 577-8502, JAPAN

(Received September 5, 2010; Revised December 2, 2010, Accepted December 12, 2010)

Abstract: The essential oil, obtained by steam distillation of flowers, leaves and stems from *Wisteria brachybotrys* Sieb.et Zucc, collected in Japan was analyzed by gas chromatography (GC) and GC-MS. The important aroma-active compounds were also detected in the oil using GC-MS/Olfactometry (GC-MS/O) and aroma extraction dilution analysis (AEDA). As a result, sixty-eight compounds from flowers of *W. brachybotrys*, accounting for 96.3%, were identified, and benzyl cyanide (31.7%), palmitic acid (8.7%), and (*Z*)-γ-bisabolene (8.4%) as the main compounds. Thirty compounds from leaves, accounting for 97.3%, were identified, and phytol (46.0%), palmitic acid (8.2%), and nonanal (5.7%) as the main compounds. Twenty-eight compounds from stems, accounting for 98.7%, were identified, and geraniol (32.8%), linalool (22.1%), and nerol (10.4%) as the main compounds. A preliminary analysis by GC-MS and using Kovats' retention indexes, lead to characterize and quantify the oil constituents, while GC-MS/O was then applied for the identification of the main odorants. By the incremental dilution method (AEDA), applied to the GC-MS/O technique, the flavor dilution (FD) factor was obtained. To our knowledge, the composition of these parts of essential oils is described here for the first time, both from the chemical and olfactometric viewpoints.

Keywords: *Wisteria brachybotrys*; essential oil; benzyl cyanide.

1. Introduction

The edible wild plant describes that grow naturally in the wild. It has been used of a wide range of native ingredients in Japan, for it has characteristic flavor different from the vegetable.

* Corresponding author: E-Mail: miyazawa@apch.kindai.ac.jp, Phone: +81-6-6721-2332; Fax: +81-6-6727-4301.

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 03/15/2011 EISSN:1307-6167

Therefore, many species of the edible wild plant has been used as traditional Japanese food. In addition, the edible wild plant was found to have high antioxidant activities [1]. It is often cultivated in recent years. Consequently, it is regard as familiar and healthy food. Therefore, we have been investigated components and characteristic odor of the essential oil from the edible wild plant and medicinal plant [2-12].

The edible wild plant *Wisteria brachybotrys* Sieb.et Zucc is a fallen leaves-related creeper belonging the family Leguminosae and can be observe in all over Japan. *W. brachybotrys* lets a purple flower bloom from April to June and gives off a refined good fragrance. In addition, it not only enjoys the sight in the Tohoku region but also the sprouts and the stems are widely eaten as a boiled green, a tempura, and a vinegared dish. However, there is no report of the volatile components of *W. brachybotrys*.

This disadvantage of the static technique can be overcome, when the odorants present in the food are first screened by an aroma extraction dilution analysis (AEDA) and are subsequently identified. The odor qualities of most of the components are known from the AEDA, these odorants are easily recognized by capillary gas chromatography-mass spectrometry-olfactometry (GC-MS/O) of the essential oils. The concept of AEDA was applied to indicate the compounds that are mainly responsible for the odors of *W. brachybotrys*.

The purpose of this study was to investigate and compare the chemical compositions of essential oil from flowers, leaves, and stems of *W. brachybotrys*. To the best of our knowledge, an investigation of the essential oil of *W. brachybotrys* has not been reported to date.

2. Materials and Methods

2.1. Plant Material

Wisteria brachybotrys were harvested from Fukushima prefecture of Japan in June 2008.

2.2 Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS was carried out with a Hewlett-Packard 6890/ Hewlett-Packard 5973 instrument. GC conditions were equipped on capillary column (HP-5MS 30 m × 0.25 mm, film thickness 0.25 μm). On HP-5MS, the column temperature was programmed from 40-260˚C at a rate of 4˚C /min and held at 260˚C for 5 min. The injector and detector temperatures were 270 and 280˚C, respectively. The flow rate of the carrier gas (He) was 1.8 mL/min. The detector interface temperature was set at 280˚C with the actual temperature in the MS source reaching approximately 230˚C and the ionization voltage 70 eV. Split ratio was 1:10. Acquisition mass range was 39-450 amu. Relative percentages were calculated from TIC automatically by the computerized integrator.

Identification of the oils components were carried out by a comparison of their relative retention times to those of authentic samples or by a comparison of their Kovats' retention indexes (KI) relative to the series of C_8-C_{29} *n*-hydrocarbons. Computer matching against commercial (NIST 98, MassFinder4, and AromaOffice) and homemade library mass spectra made of pure substances and components of known oils and MS literature data was also used for identification.

Table 1. Composition of essential oils from flowers, leaves, and stems of *W. brachybotrys*.

KI	compounds	percent composition (%)[a]		
		flowers	leaves	stems
796	1-octene	0.7	-	-
800	octane	0.7	-	-
851	(2E)-hexenal	0.1	-	-
852	(3Z)-hexenol	0.9	4.8	2.3
865	hexanol	0.6	0.5	t
903	heptanal	0.2	-	-
907	3-(methylthio)-propanal	0.2	-	-
911	2,3-dimethylpyrazine	0.2	-	-
961	benzaldehyde	0.2	-	-
979	1-octen-3-one	0.4	-	-
981	1-octen-3-ol	0.2	3.7	9.3
988	3-octanone	0.8	-	-
993	2-pentylfuran	0.1	-	t
994	3-octanol	0.7	-	1.2
999	2-carene	-	1.2	-
1004	octanal	0.3	-	-
1006	(3Z)-hexenyl acetate	-	t	0.9
1027	2-ethyl-1-hexanol	t	t	0.7
1039	benzyl alchool	2.9	-	-
1046	phenyl acetaldehyde	0.5	-	-
1050	(*E*)-β-ocimene	0.2	-	-
1058	unknown	0.7	-	-
1066	(2*Z*)-octenol	-	t	0.4
1071	(2*E*)-octenol	0.3	-	-
1074	octanol	0.6	1.6	1.8
1100	linalool	2.9	5.0	22.1
1103	nonanal	7.5	5.7	4.8
1118	benzylhydrazine	2.1	-	-
1120	2-phenylethanol	2.1	-	-
1154	benzyl cyanide	31.7	-	-
1161	unknown	0.2	-	-
1163	(2*E*)-nonenal	0.2	-	-
1169	nonanol	0.3	1.9	0.5
1195	α-terpineol	0.5	1.4	6.5
1203	decanal	0.5	-	0.3
1219	2,3-dihydrobenzofuran	0.7	2.2	-
1227	nerol	1	2.4	10.4
1239	*trans*-chrysanthenyl acetate	t	t	0.9
1244	neral	0.2	t	t
1255	geraniol	3.0	5.5	32.8

Table 1. Continued.

KI	compounds	percent composition (%)[a]		
		flowers	leaves	stems
1269	geranial	t	t	1.5
1273	citronellylformate	0.2	-	-
1279	unknown	0.3	-	-
1287	unknown	-	t	0.3
1299	indole	2.4	-	-
1303	unknown	0.2	-	-
1306	4-vinyl-*o*-guaiacol	0.2	1.0	-
1309	undecanal	0.1	-	-
1320	unknown	-	-	0.6
1346	methylanthranilate	1.4	-	-
1412	(*E*)-β-damascone	-	0.8	t
1468	unknown	-	1.3	-
1475	unknown	0.2	-	-
1483	(*E*)-β-ionone	0.1	0.8	t
1497	α-zingiberene	0.3	-	-
1505	(*E*,*E*)-α-farnesene	-	0.8	t
1508	unknown	t	t	-
1514	(*Z*)-γ-bisabolene	8.4	-	-
1524	unknown	0.2	-	-
1567	(*E*)-nerolidol	0.3	-	-
1632	unknown	0.3	-	-
1675	unknown	0.2	-	-
1727	(*E*,*E*)-farnesol	2.6	-	-
1766	myristic acid	0.2	-	-
1770	benzylbenzoate	0.2	-	-
1847	6,10,14-trimethyl-2-pentadecanone	0.2	-	-
1867	pentadecylic acid	0.2	-	-
1942	unknown	0.5	-	-
1949	isophytol	0.3	-	-
1960	palmitic acid	8.7	8.2	1.5
1963	unknown	0.3	t	t
2033	unknown	0.3	-	-
2100	heneicosane	0.1	-	-
2109	phytol	0.9	46.0	-
2147	linoleic acid	1.4	-	-
2154	linolenic acid	2.1	3.0	0.8
2235	unknown	0.3	-	-
2300	tricosane	0.8	-	-
2500	pentacosane	0.7	1.3	t
2700	heptacosane	0.5	-	-
	total	100	100	100

[a] Percent composition percentages are calculated in GC on HP-5MS column. t: trace (<0.1%)

2.3 Gas Chromatography-Mass Spectrometry-Olfactometry (GC-MS/O)

GC-MS/O was carried out using a Hewlett-Packard 6890/ Hewlett-Packard 5973-Olfactory Detection Port 2. GC condition was equipped on a capillary column (HP-5MS 30 m × 0.25 mm, film thickness 0.25 μm). The column temperature was programmed from 40-260˚C at a rate of 4˚C /min and held at 260˚C for 5min. The injector and detector temperatures were 270 and 280˚C, respectively. The flow rate of the carrier gas (He) was 1.8 mL/min. The detector interface temperature was set at 280˚C with the actual temperature in the MS source reaching approximately 230˚C and the ionization voltage 70 eV. Split ratio was 1:10. Acquisition mass range was 39-450 amu. The Chemstation software acquired two channel signals simultaneously, one channel for MS, and other channel from the olfactometer signal board. A signal sniffer, the author, recorded the aroma character manually.

2.4 Aroma Extract Dilution Analysis (AEDA)

The flavor dilution (FD)-factor of the odorants in the essential oil was determined by aroma extract dilution analysis (AEDA) of the following dilution series [11, 12]. The highest dilution was defined as FD-factor 1 (7.0 mg/ml). The oil was stepwise diluted (1+1, v/v) by addition of diethyl ether. Aliquots were then analyzed by GC-MS/O on the capillary column HP-5MS. The highest dilution at which an individual component could be detected was defined as the FD-factor for that odorant.

3. Results and Discussion

The essential oils collected by steam distillation from flowers, leaves and stems of *W. brachybotrys* were obtained in 0.0187, 0.0057, and 0.0037% yields (w/w), respectively. Gas chromatograms of these oils showed the presence of 80 compounds (Table 1). As a result, 68 compounds from flowers, 30 compounds from leaves, and 28 compounds from stems of *W. brachybotrys* accounting for 96.3, 97.3, and 98.7% were identified, respectively. The classification of these oils on the basis of structure type is summarized in Table 2. Distinct differences in the qualitative and quantitative from these oils were observed.

The essential oil from flowers of *W. brachybotrys* contained nine monoterpenes, four sesquiterpenes, two diterpenes, 12 aliphatic compounds, four aromatic compounds, and three miscellaneous compounds. The major constituents were benzyl cyanide (31.7%), palmitic acid (8.7%), (*Z*)-γ-bisabolene (8.4%), nonanal (7.5%), and geraniol (3.0%). A benzyl cyanide was detected from chemical composition of *Wisteria* species for the first time.

The essential oil from leaves contained seven monoterpenes, a sesquiterpene, a diterpene, 24 aliphatic compounds, and an aromatic compound. The major constituents were phytol (46.0%), palmitic acid (8.2%), nonanal (5.7%), geraniol (5.5%), and linalool (5.0%).

The essential oil from stems contained eight monoterpenes, a sesquiterpene, 24 aliphatic compounds, and a miscellaneous compound. It was determined to be rich in monoterpenes. The major constituents were geraniol (32.8%), linalool (22.1%), nerol (10.4%), 1-octen-3-ol (9.3%) and α-terpineol (3.0%). A comparison of the composition, except for phytol, of leaves and stems oils showed similar.

In the AEDA of these oils, FD factors were based on the AEDA evaluations of one panelist, because the responses of all three panelists were very similar: the differences in FD factors were not greater than 2 between the panelists.

Table 2. Classification of essential oil composition of *W. brachybotrys*.

compounds	percent composition (%)		
	flowers	leaves	stems
Aliphatics	51.1	95.3	98.6
terpenoids			
monoterpenes	7.8	15.6	73.2
sesquiterpenes	11.6	1.7	t
diterpenes	1.2	46.1	-
miscellaneous			
hydrocarbones	3.5	1.4	t
alcohols	3.6	12.5	16.1
aldehydes	9.1	5.8	5.1
ketones	1.5	0.9	t
acids	12.6	11.3	2.3
esters	0.2	t	1.9
Aromatics	44.6	3.5	-
Miscellaneous	0.5	-	t
Unknown	3.8	1.2	1.4

t: trace (< 0.1%)

Table 3. Odor-active compounds in essential oils from flowers, leaves, and stems of *W. brachybotry*.

KI	compounds	odor	FD factor		
			flowers	leaves	stems
851	(2*E*)-hexanal	green	1	-	-
852	(3*Z*)-hexenol	green	1	32	16
865	hexanol	herbal	4	-	-
903	heptanal	green	1	-	-
907	3-(metylthio)-propanal	powdery	32	-	-
981	1-octen-3-ol	earthy	4	4	8
1058	unknown	citrus	2	-	-
1100	linalool	floral	32	4	16
1103	nonanal	oil-like, aldehydic	2	16	16
1195	α-terpineol	floral	2	1	1
1244	neral	floral	8	-	-
1255	geraniol	floral	-	4	16
1287	unknown	sour	4	-	-
1306	4-vinyl-*o*-guaiacol	medicinal	8	4	-
1412	(*E*)-β−damascone	fruity	-	4	2
1508	unkown	woody	-	16	16

KI: Kovats' retention indexes o HP-5MS capillary column.

Aroma-active compounds detected in these oils and their aroma properties given in Table 3. 3-(Methylthio)-propanal and linalool were the most intense aroma-active compounds in flowers oils, with FD factors of 32. (3*Z*)-Hexenal and nonanal were identified as key compounds contributing to the aroma of leaves oils, with relatively high FD factors of 32 and 16, respectively. The FD factoes of (3*Z*)-hexenal, nonanal, linalool, and geraniol were high, 16, in stems oils.

In conclusion, the major components of essential oil from flowers of *W. brachybotrys* were benzyl cyanide (31.7%), palmitic acid (8.7%) and (*Z*)-γ-bisabolene (7.6%), from leaves oil were phytol (46.0%), palmitic acid (8.2%), and nonanal (5.7%), from stems oil were geraniol (32.8%), linalool (22.1%), and nerol (10.4%). The characteristics compound, benzyl cyanide, was contained only in the oil from flower part. In addition, the impotant odor-active compounds in essential oils from flowers, leaves, and stems of *W. brachybotrys* were identified by GC-MS/O and AEDA.

References

[1] K. Umegaki, I. Kajiki (2007). Component analysis of cultivated Shi-O-de (an edible wild plant). *Eiyogaku Zasshi* **65**, 81-83.
[2] M. Miyazawa, H. Kameoka (1988). Volatile flavor components of crude drug. V. Volatile flavor components of *Zingiberis Rhizoma* (*Zingiber officinale Roscoe*), *Agric. Biol. Chem.* **52**, 2961-2963.
[3] M. Miyazawa, H. Kameoka (1977). The constituents of the Essential Oil from *Eupatorium japonica* Thunb, *Yakugaku Zassi* **97**, 120-122.
[4] M. Miyazawa, H. Kameoka (1978). The Essential Oil of *Artemisia capillaries, Phytochemistry* **16**, 1054-1057.
[5] M. Miyazawa, H. Ikeda, H. Kameoka (1978). The constituents of the Essential Oil from *Oenothera biennis* L, *Nippon Nogei Kagaku Kaishi* **52**, 449-455.
[6] M. Miyazawa, H. Ikeda, H. Kameoka (1979). The constituents of the Essential Oil from *Oenothera stricta* Ledeb. ex Link, *Nippon Nogei Kagaku Kaishi* **53**, 349-354
[7] M. Miyazawa, H. Kameoka (1979). Studies on chemical constituents of naturalized plants of Japan. VI. The constituents of the Essential Oil from *Erigeron annuus, Agric. Biol. Chem.* **43**, 2199-2201.
[8] M. Miyazawa, H. Kameoka (1988). Volatile flavor components of crude drugs. IV. Volatile flavor components of Puerariae Radix (*Pueraria lobata* Ohwi), *Agric. Biol. Chem.* **52**, 1053-1055.
[9] M. Miyazawa, E. Horiuchi, J. Kawata (2007). Components of the Essential Oil from *Matteuccia struthiopteris, J. Oleo Sci.* **56**, 457-461.
[10] M. Miyazawa, E. Horiuchi, J. Kawata (2007). Components of the Essential Oil from *Adenophora triphylla* var. *japonica, J. Essent. Oil Res.* **20**, 125-127.
[11] M. Miyazawa, Y. Utsumi, J. Kawata (2008). Comparison of the Essential Oils from Wild type and Cultivar type of *Hosta sieboldiana*, *J. Essent. Oil Bear. Plants* **11**, 413-422.
[12] M. Miyazawa, Y. Utsumi, J. Kawata (2009). Aroma-active compounds of *Elatostema laetevirens* and *Elatostema umbellatum* var. *majus*, *J. Oleo Sci*. **58**, 163-169.