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Chemical Composition of the Essential Oil of *Perilla frutescens*

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Essential oil constituents of the major chemotypes of *Perilla frutescens*, such as perillaldehyde [PA] type, elsholtziaketone [EK] type, perillaketone [PK] type, citral [C] type, perillene [PL] type and phenylpropanoid [PP] type, were examined by GC/MS. Seventy-one compounds were detected and identified.

Keywords: *Perilla frutescens*; essential oil; chemotype; GC-MS

Perilla frutescens is a medicinal herb and is also a popular vegetable widely cultivated in the East Asia. It includes many chemical variants of the essential oil composition and they are classified into the following 6 chemotypes: (1) perillaldehyde [PA] type; (2) elsholtziaketone [EK] type; (3) perillaketone [PK] type; (4) perillene [PL] type; (5) citral [C] type; and (6) phenylpropanoid [PP] type. Biosyntheses of the essential oil constituents of these chemotypes were demonstrated to be under the control of several genes.[1)-9)] Most of the studies on the essential oils were carried out by using GC equipped with the glass column.[10)-12)] In this paper, we report on the detailed analysis of the steam-distilled essential oils of the major chemotypes of *P. frutescens* by capillary gas chromatography and GC-MS.

MATERIALS AND METHODS

Plant materials *Perilla* strains kept true by repetitive self-pollination were grown in the field of the Experimental Station for Medicinal Plants, Graduate School of Pharmaceutical Sciences, Kyoto University, and the fresh leaves from the plants were collected in September for the experiment.

Extraction Fresh leaves (*approx.* 200 g) were steam-distilled with 750 ml of the ion exchanged water for 2 hours by using the apparatus designated in the Japanese Pharmacopoeia XIII.[13)] The extracted oil was preserved in an airtight container at -30 ℃.

Chromatography GC analysis was carried out with Hitachi G-5000 under the following operation conditions: column: fused silica capillary column, TC-wax (Hewlett Packard), 60 m×0.25 mm, df = 0.25 mm; column temperature program: 100-200℃, increasing at 3℃/min, ending at 200℃ for 15 minutes; injector: 180℃, split ratio = 1/100; detector: 180℃, FID; carrier gas: helium, 37 cm/sec; make up gas: nitrogen; column head pressure: 320 kPa; injection volume: 1 μl.

GC-MS analysis was performed on Hewlett Packard 5890 series II gas chromatograph connected with AUTOMASS 50 (JEOL), operation conditions were as follows: column: fused silica capillary column, TC-wax (Hewlett Packard), 60 m×0.25 mm, df = 0.25 mm; column temperature: 100-200℃ increasing at 3℃/min, ending at 200℃ for 20 minutes; injector: 180℃, split; carrier gas: helium, 30 cm/sec; column head pressure: 180 kPa; injection volume: 0.5 μl; ionization energy: 70 eV; ion source temperature: 200℃. Chemical components were identified by comparing their retention times and mass spectra with those of authentic samples and/or with reference data [1),14)] or by comparing their mass spectra with those in MS data library (NBS library). The relative amount of each component was determined by calculating the peak area of the TIC chromatogram.

RESULTS AND DISCUSSION

Table 1 shows the identified constituents and their relative content in the essential oils of the strains of *P. frutescens* examined. The compounds are listed in the table in the order of elution from the GC column.

(1) PA type

The PA type of perilla is the chemotype of perilla designated in the Japanese Pharmacopoeia and its principal compound, perillaldehyde, is a distinctive compound found exclusively in *Perilla frutescens*. The content of perillaldehyde in the essential oil of the PA type turned out to be 48.36-56.13% and that of limonene was 15.98-18.85%. These data agreed with those reported in the previous papers.[10)-12)] L-Menthol reported by Ito[10),14)] and Morisada[11)] was, however, not detected in the strains tested.

(2) EK type

The principal constituents of the EK type are elsholtziaketone and naginataketone. Nishizawa et al. grouped this type into 4 subtypes according to the content ratios between elsholtziaketone and naginataketone.[6)] Recently, a new compound, shisofuran, was identified in the essential oil of a wild species of *Perilla*,[15)] and this compound was shown to be contained in the oil of the EK type by the present assay, though Nishizawa's GC condition did not detect shisofuran and elsholtziaketone separately. Thus, the data reported by Nishizawa et al. that more than 95% of the monoterpenoids in the essential oil of No. 79 was composed of elsholtziaketone, should be revised as follows: the content of elsholtziaketone in No. 79 is approximately 30% and that of shisofuran is 41% (Table 1). Yet, shisofuran is so sensitive to both light and heat that the compound may have been mostly decomposed during steam-distillation.[15)] In order to estimate the shisofuran and elsholtziaketone contents in fresh material, leaves of No. 79 were extracted with diethyl ether at 4℃ and the ether extract was analyzed by GC equipped with a packed column. As a result, content of shisofuran and elsholtziaketone turned out to be 51.8% and 11.6%, respectively.

(3) PK type

Koezuka et al. classified the PK type into the following two subtypes; the PK-I type, containing much perillaketone and little (< 1%) isoegomaketone, and the PK-II type, containing perillaketone and isoegomaketone in an approximately equal amount.[4)] Among the PK type strains described in this paper, No. 11 and No. 5526 were of the PK-I type, and No. 6 and No. 63 of the PK-II type. Perillaketone and isoegomaketone are supposed to be synthesized from perillene via a common precursor egomaketone,[4)] however, egomaketone was not detected in the PK type in this experiment, and perillene was detected in a small amount in the PK-II type but not in the PK-I type.

(4) PL type

The predominant constituent of the PL type is perillene, which accounts for nearly 90% of the total essential oil. A small amount of *trans*-citral, a possible precursor of perillene, was detected but a further oxidized compound, perillaketone, was not detected.

(5) C type

The C type, whose principal constituent is citral, and which is common among *P. citriodora*, the wild species, but had not been reported among other *Perilla* species. The C type plants were generated for the first time among the F_2 progenies of the crossing of a PK type plant with a PL type plant of *P. frutescens*, and bred true to the C type in the cultivated species.[8)] Most of the constituents detected in this C type were common with those in the PL type, though, their contents varied.

(6) PP type

The PP type of *P. frutescens* are known to be classified into the following 3 subtypes according to the composition of their phenylpropanoids; the PP-m (myristicin) type, the PP-dm (dillapiole, myristicin) type, and the PP-em (elemicin, myristicin) type.[3)] Among the strains examined the present experiment, No.s 12, 10, 16 and 25 were of the PP-m, PP-em, PP-md and PP-emd types, respectively.

The essential oil extracted from the leaves of the PP type of *P. frutescens* does not contain monoterpenoids generally, but a small amount of monoterpenoids such as

limonene, perillaldehyde or perillaketone were detected in almost all the PP type strains examined. These monoterpenoids were supposed to be contained in the petioles, where the essential oil constituents were different from those in the leaves.[16]

Sesquiterpene compounds were detected in all the chemotypes examined, however, the relative content of β-caryophyllene was considerably high in the PP type strains (31.87-10.05%) than in the monoterpene type strains (9.54-1.06%).

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Table 1. Composition of the essential oils of *Perilla frutescens*

	Compound	(Strain No.) (Chemotype)	9 PA	32 PA	5343 PA	79 EK	1841 EK	6 PK	11 PK	63 PK	5526 PK	1864 PL	5536 PL	5254 C	10 PP-em	12 PP-m	16 PP-md	25 PP-emd
1.	α-pinene		1.31	0.80	1.05										0.18		0.42	0.60
2.	hexanal									0.12		0.10					0.18	0.45
3.	camphene		0.13												0.13			
4.	β-pinene		1.27	0.82	1.32										tr		0.14	0.20
5.	β-myrcene		0.21		0.19	0.18				tr		0.20	0.15	0.15			tr	0.17
6.	ψ-limonene		0.20	0.15	0.20													
7.	limonene		18.19	15.98	18.85					tr		tr			1.69		2.25	1.13
8.	1,8-cineol				0.22													
9.	leaf aldehyde		0.33							0.56	0.33	0.26	0.19		0.54		0.41	0.80
10.	2-carene		0.14	0.10	tr													
11.	1,2,4-trimethyl cyclopentane				tr													
12.	4-hexene-1-ol acetate																	0.15
13.	*n*-hexyl formate															tr		0.20
14.	1-methylbutyl oxirane																tr	
15.	6-methyl-5-heptene-2-one		tr		tr	0.13						0.47		4.29	tr	tr		
16.	3-octanol			0.49	0.55	0.23	0.35	0.14	0.26	0.30	0.19	0.10	0.16	0.40	0.31	0.77	0.42	
17.	2-hexene-1-ol		tr									tr			tr		tr	0.19
18.	cinerone					2.99								tr				
19.	perillene		tr		tr	tr		0.14		0.38		83.03	94.86	24.41	tr		tr	
20.	1-octen-3-ol		1.03	1.43	3.42	1.04	1.71	0.34	1.46	3.21	1.31	0.64	1.62	1.23	3.74	5.14	4.18	2.31
21.	unknown					0.59	0.11											
22.	citronellal													0.12				
23.	ylangene		tr		tr					tr				0.10	tr		tr	0.11
24.	β-bourbonene						0.33			tr								
25.	linalool		1.95	1.86	2.24	3.86	7.07	1.14	1.33	1.74	1.61	1.89	0.55	3.01	0.16	0.47	0.29	0.24
26.	benzaldehyde		0.43	2.44		0.27				0.13		tr	0.24	0.50	tr	tr	0.55	0.87
27.	elemene									tr		tr			tr			0.12
28.	4-terpineol		tr															
29.	β-caryophyllene		5.43	6.03	9.31	4.20	8.05	4.20	3.53	5.48	1.06	7.01	tr	9.54	22.63	25.53	10.05	24.67
30.	shisofuran					40.78	tr											
31.	elsholtziaketone					30.55	30.14											
32.	β-farnesene						0.27						0.56		3.07	2.10	2.82	3.20
33.	acetophenone									tr								
34.	α-caryophyllene		0.42	0.48	0.71	0.29	0.96	0.33	0.37	0.39	tr	0.83		2.47	2.07	2.50	0.77	1.72
35.	*cis*-citral		tr											8.35				
36.	methyl geranate					0.18	0.10	0.18		0.14		0.30	0.17	1.07				

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37.	α-terpineol			0.31	0.24						tr						tr	0.10
38.	germacrene D			0.63	0.66	0.34	0.47	0.31	0.53	0.50	0.16	0.29	0.26	1.53	0.38	0.68	0.25	0.50
39.	α-farnesene		3.80	6.54	5.86	3.79		3.85	5.09	5.05	1.04				6.42		11.87	7.72
40.	geranyl acetate											tr		0.69				
41.	*trans*-citral		tr			tr						0.77		15.10				
42.	elixene		0.26	0.35		0.25		0.29				0.61		0.63	1.59	0.43	1.08	2.07
43.	unknown		tr	0.25	0.24												0.76	tr
44.	naphthalene		0.15	0.26	0.14	0.30	tr	0.16	0.15	0.31	tr				0.28	0.33	0.36	0.60
45.	citronellol formate			0.18								1.06		9.60				
46.	δ-cadinene									tr					tr			tr
47.	6-octene-1-ol,7-methyl-3-methylene											tr						
48.	methyl salicylate		0.10	0.22			0.25		0.13		0.32	tr	0.10			0.65		
49.	nerol											1.03		8.58				
50.	perillaldehyde		56.13	50.42	48.36										2.96		4.46	1.96
51.	perillaketone						tr	53.09	84.58	57.91	92.88					5.07		
52.	isogeraniol											0.13		2.80				
53.	damascenone										tr							
54.	geraniol		tr		tr					tr	tr	tr		0.91	tr		tr	
55.	geranylacetone											tr						
56.	naginataketone					4.20	30.14											
57.	shisool		0.18	6.41	3.59										0.15		0.20	0.13
58.	patchoulene																tr	tr
59.	piperitenone											tr						
60.	isoegomaketone							33.44		21.49								
61.	caryophyllene oxide		tr	0.35	0.11	0.38	0.13	0.16		0.11	0.12	tr		0.24	tr	0.14		
62.	perillyl alcohol		0.32	1.16	0.72												tr	
63.	methyl eugenol														tr	tr		
64.	nerolidol		0.11	0.47	0.22	0.10	0.30	0.22	0.13	0.13	tr	0.17	0.14	0.52	0.14	0.47	0.18	0.42
65.	phytol		tr				tr		0.86						tr			
66.	anethol												0.50					1.56
67.	isoeugenol		0.16	0.11	0.15	tr	0.15		0.41		0.27	0.19	0.10	0.22	0.26	0.35	tr	0.22
68.	1,2,3,4,tetramethoxy-5-(2-propenyl)-benzene																0.14	3.24
69.	elemicin														35.87		tr	28.30
70.	myristicin									tr					16.32	54.51	4.57	2.97
71.	dillapiol																53.20	9.01

tr= < 0.1%