

Flavor Components in the Bellflower Roots (*Platycodon glaucum* Nakai)

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도라지 뿌리의 향기성분에 관하여

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Abstract

Flavor components were trapped by simultaneous steam distillation-extraction method for investigating it in the bellflower roots and fractionated into four groups such as a neutral, a basic, a phenolic and an acidic fraction. An acidic fraction methylated with diazomethane solution and three others were analyzed by GC and GC-MS equipping a fused silica capillary column, and S-containing compounds in these were detected with a flame photometric detector (FPD). The total of one hundred and three compounds from the bellflower roots were identified: they were 6 aliphatic hydrocarbons, 10 aromatic hydrocarbons, 2 terpene hydrocarbons, 12 alcohols, 8 terpene alcohols, 17 aldehydes, 3 terpene aldehydes, 5 ketones, 5 esters, 3 furans, 2 thiazoles, 2 lactones, 2 sulfides, 9 phenols, 12 carboxylic acids and 5 others. The greater part of the others except carboxylic acids were identified from a neutral fraction of which was assumed to be indispensable for the reproduction of bellflower root odor in a sensory evaluation. As a result of a sensory evaluation, 1-hexanal, trans-2-hexenal, 1-hexanol, cis-3-hexenol, trans-2-hexenol, 1-octen-3-ol and so forth identified in a neutral fraction were considered to be the key compounds of grass-like odor in the bellflower roots.

요 약

도라지 뿌리의 향기성분을 규명하고자 도라지 뿌리의 향기성분을 상압 수증기 증류법으로 포집하여 중성, 염기성, 약산성 및 산성부분으로 분획하였다. Diazomethane 법으로 methyl ester화한 산성부분을 위시킨 4부분은 모세관 column을 장치한 GC 및 GC-MS로 분석하였으며 함황화합물은 FPD 검출기로서 검출하였다. 도라지 뿌리로부터 aliphatic hydrocarbon류 6종, aromatic hydrocarbon류 10종, terpene hydrocarbon류 2종, alcohol류 12종, terpene alcohol류 8종, alde-

hyde류 17종, terpene aldehyde류 3종, ketone류 5종, ester류 5종, furan류 3종, thiazole류 2종, lactone류 2종, sulfide류 2종, phenol류 9종, 산류 12종 및 기타 5종을 위시하여 총 103종의 향기 성분이 동정되었다. Carboxylic acid를 제외한 향기성분은 대부분 중성부분에서 동정되었고, 관능 검사의 결과에서도 중성부분이 도라지 뿌리의 향기를 재현하는데 필요불가결한 것으로 나타났다. 관능검사의 결과, 중성부분에서 동정된 1-hexanal, trans-2-hexenal, 1-hexanol, cis-3-hexenol, trans-2-hexenol, 1-octen-3-ol 등은 도라지 뿌리의 꽃냄새의 주성분인 것으로 생각된다.

Introduction

The authors are investigating on the flavor components of the bellflower roots since 1981. Several nonvolatile components, such as free amino acids, vitamin C, phytosterols and so forth concerning with the major taste components in the flavor constituents of the bellflower roots have been reported till recently¹⁻³⁾. In the case of Korea, the consumption of the bellflower roots having their peculiar flavor and the effect of a chinese medicine is annually increasing. For reasons being of wide use, the cultivated bellflower roots are producing on a nationwide scale so that production of a wild one can not satisfy domestic needs.

In raw material, the bellflower roots have grass-like odor and an undesirable taste, so that they are not suitable for the table. Usually, they need further treatment, such as cooking or processing in general use for food. Their undesirable odor or taste have been eliminated by immersing themselves in a hot bath for many hours or washing with running water after pickled in salt. In a chinese medicine, dehydrated bellflower roots have been largely used for treating tonsillitis, suppurative bronchitis, pharyngitis and so forth as expectorant and a drainage drug.

Although pharmacological researches on the bellflower roots have been much performed till recently, the reports on their volatile components are seldom shown. In this paper, the authors deal with the separation and identification of volatile components in each fraction by high-resolution GC and GC-MS equipping

with a fused silica capillary column.

Materials and Methods

Materials

The three-year-old and the cultivated bellflower roots used in this research was purchased from Oshigae market in Dongrae-Ku, Pusan, on November 5th, 1981. Fresh bellflower roots were washed with running water to remove unclean substances after purchasing, and then these were preserved in a freezer at -20°C before the time of experiment.

Preparation and fractionation of the whole steam volatile concentrate

One kilogram of the crushed bellflower roots was placed in a 10 liter glass vessel and steam distillation was performed for 2 hrs under atmospheric pressure. The steam-distillate was saturated with sodium chloride and then extracted with diethyl ether. The ether extract was concentrated at 36~38°C under atmospheric pressure. The whole steam volatile concentrate was obtained in a yield of 1.7g from 10kg of the bellflower roots. The whole steam volatile concentrate was fractionated into a neutral, a basic, a phenolic and an acidic fraction by the same methods as shown in Figure 1. Each fraction was concentrated by a similar method as mentioned above. An acidic fraction was treated with diazomethane for methylation of the acids. These fractionated volatile components were analyzed by GC and GC-MS.

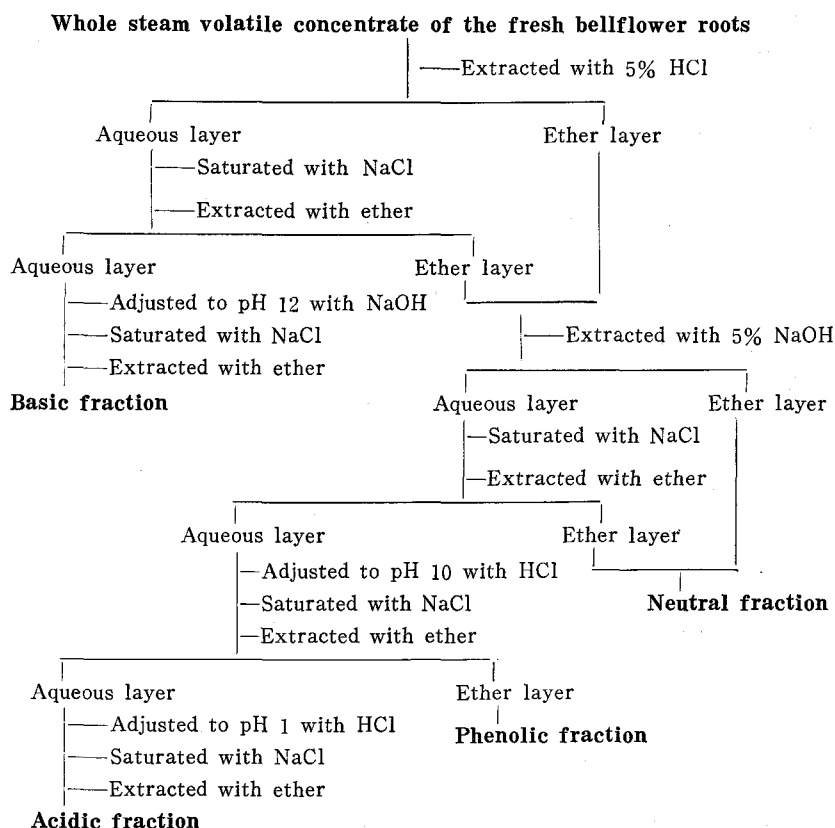


Fig. 1. Fractionation of Whole Steam Volatile Concentrate of the Fresh Bellflower Roots

Gas chromatography

Each fraction obtained from the whole steam volatile concentrate was analyzed with a Shimadzu Model 7A Gas Chromatograph equipped with a flame ionization detector (FID) and a flame photometric detector (FPD). A fused silica capillary column (50m × 0.25mm i.d.) coated with PEG 20M was used. The column oven temperature was programmed from 60°C to 190°C at 4°C/min. The injection port and detector temperatures were kept at 200°C. Nitrogen was used as the carrier gas at a flow rate of 1ml/min with a split ratio of 1:39.4.

Gas chromatography-mass spectrometry

GC-MS spectra were recorded with a Jeol

Model DX-300 Mass Spectrometer equipped with RIC detector. The ionization voltage was 70eV and the ion source temperature was kept at 170°C. A fused silica capillary column (50m × 0.25mm i.d.) coated with PEG 20M was used. The column oven temperature was programmed from 60°C to 170°C at a rate of 4°C/min and the injection port temperature was 170°C. Helium was used as the carrier gas at a flow rate of 1ml/min with a split ratio of 1:10.

Results and Discussion

Yield and odor of each fraction obtained from whole steam volatile concentrate of the fresh bellflower roots

Table 1 shows the yield and odor description

Table 1. Yield and Odor of Each Fraction obtained from the Whole Steam Volatile Concentrate of the Fresh Bellflower Roots

Fraction	Yield(g)*	Odor**
NF	1.45	Oily, fresh bellflower root-like
BF	0.01	Burnt
PF	0.03	Medicinal, guaiacol-like
AF	0.09	Rancid or sweat-like
UR	0.12	

NF, neutral fraction; BF, basic fraction;
 PF, phenolic fraction; AF, acidic fraction;
 UR, unrecovery.

* Yield from 10kg of the fresh bellflower roots.

** The ethereal solution of each fraction was absorbed on a filter paper and the filter paper was air-dried to remove the solvent and then subjected to the organoleptic test by 10 members of our department.

of each fraction obtained from the whole steam volatile concentrate of the fresh bellflower roots. Most of the volatile components were present in the neutral fraction and the odor of the neutral fraction was exactly similar to that of the whole steam volatile concentrate. The neutral fraction of the fresh bellflower roots had a fresh bellflower root-like aroma with an oily odor. The others except for the neutral fraction showed very low yields. A basic, a phenolic

and an acidic fraction had a burnt, medicinal or guaiacol-like and rancid or sweat-like odor, respectively. These results indicate that important components of the fresh bellflower root odor were present in the neutral fraction. As a result of organoleptic test shown in Table 2, there would be a close relationship between the neutral fraction and the whole steam volatile concentrate. The neutral fraction would be considered to be indispensable for the reproduction of the fresh bellflower root odor.

Identified volatile components

Volatile components identified in the fresh bellflower roots are given in Table 3. The total of one hundred and three compounds, such as 6 aliphatic hydrocarbons, 10 aromatic hydrocarbons, 2 terpene hydrocarbons, 12 alcohols, 8 terpene alcohols, 17 aldehydes, 3 terpene aldehydes, 5 ketones, 5 esters, 3 furans, 2 thiazoles, 2 lactones, 2 sulfides, 9 phenols, 12 acids and 5 others were identified. By comparing the neutral fraction with the whole steam volatile concentrate, most volatile components except for acids indicated a similar relationship between the former and the latter. Synthetic antioxidant for food such as BHT (peak 123) would be considered to be a contaminant originated from diethyl ether used as solvent. Volatile components identified in each

Table 2. Organoleptic Contribution of Each Fraction on the Whole Steam Volatile Concentrate by Flavor Profile Method

Fraction	Score*										Average
	1	2	3	4	5	6	7	8	9	10	
NF	4	5	4	3	4	5	5	3	4	5	4.2
BF	2	3	2	2	3	3	1	3	3	3	2.5
PF	2	2	2	1	1	2	3	2	2	2	1.9
AF	1	1	2	1	1	2	1	1	1	1	1.2

5, exactly resemble; 4, closely resemble; 3, medially resemble; 2, slightly resemble; 1, no resemble

* Scored from 1 to 5 in each fraction by 10 members of our department

Table 3. Volatile Components identified in the Steam-Distillate of the Fresh Bellflower Roots

Peak No.	Compound	Method of Identification				GC-MS
		GC				
		NF	BF	PF	AF	
	Aliphatic hydrocarbons					
24	Undecane	*				*
72	Pentadecane	*				*
96	Hexadecane	*				*
100	Heptadecane	*				*
138	Eicosane	*				*
180	Tricosane	*				*
	Aromatic hydrocarbons					
8	Benzene	*				*
15	Toluene	*	*	*	*	*
27	Ethylbenzene	*				*
28'	p-Xylene	*				*
29	m-Xylene	*				*
42	Styrene	*				*
44	p-Cymene	*				*
49	Pseudocumene	*				*
57	1, 2, 3-Trimethylbenzene	*				*
102	Naphthalene	*				*
	Terpene hydrocarbons					
34'	d-Limonene	*				*
50	Terpinolene	*				*
	Alcohols					
7	Ethanol	*	*	*	*	*
16	2-Butanol	*				*
22	1-Penten-3 ol	*				*
28	1-Butanol	*				*
34	3-Methyl-butanol	*				*
39	1-Pentanol	*				*
55	1-Hexanol	*				*
58	cis-3-Hexenol	*				*
61	trans-2-Hexenol	*				*
93	Furfuryl alcohol	*				*
119	Benzyl alcohol	*				*
121	2-Phenylethanol	*				*
	Terpene alcohols					
80	Linalool	*				*
89	Isoborneol	*				*
98	α -Terpineol	*				*
99	Borneol	*				*
115	Geraniol	*				*
139	Nerolidol	*				*
190	Farnesol	*				*
200	Phytol	*				*
	Aldehydes					
7'	3-Methyl-1-butanal	*	*	*	*	*
11	1-Pentanal	*				*
21	1-Hexanal	*				*

Peak No.	Compound	Method of Identification				GC-MS
		NF	BF	GC PF	AF	
30	2-Methyl-2-pentanal	*				*
33	3,4-Pentadienal	*				*
37	trans-2-Hexenal	*				*
48	1-Octanal	*				*
53	trans-2-Heptenal	*				*
64	trans-2-Octenal	*				*
68	2-Furfural	*				*
70	trans, trans-2,4-Heptadienal	*				*
73	Benzaldehyde	*				*
84	5-Methyl-2-furfural	*				*
92	p-Tolualdehyde	*				*
109	o-Hydroxybenzaldehyde	*				*
112	p-Hydroxybenzaldehyde	*				*
116	m-Hydroxybenzaldehyde	*				*
	Terpene aldehydes					
71	Citronellal	*				*
97	Neral	*				*
105	Geranial	*				*
	Ketones					
3	Acetone	*				*
26	3-Penten-2-one	*				*
124	β -Ionone	*				*
156	3,4-Dimethoxy-acetophenone	*				*
203	Farnesylacetone	*				*
	Esters					
3'	Ethyl formate	*				*
6	Ethyl acetate	*	*	*	*	*
137	Methyl myristate	*				*
144	Ethyl myristate	*				*
175	Methyl palmitate	*				*
	Furans					
20	2,5-Dihydrofuran	*				*
78	2-Acetylfuran	*				*
123'	4,7-Dimethylbenzofuran	*				*
	Thiazoles					
126	Benzothiazole	*	*			*
208	2-Methylthiobenzothiazole	*	*			*
	Lactones					
182	Dihydroactinidiolide	*				*
210	Coumarin	*				*
	Sulfides					
1'	Dimethyl sulfide	*	*	*	*	*
2	Methyl ethyl sulfide	*				*
	Phenols					
108	o-Hydroxyacetophenone	*		*		*
117	Guaiacol	*		*		*
135	Phenol			*		*
135'	o-Cresol			*		*
160	Methylisoeugenol			*		*

Peak No.	Compound	Method of Identification				
		NF	BF	GC		GC-MS
				PF	AF	
165	Eugenol			*		*
173	4-Vinylguaiacol			*		*
204	4-Vinylphenol			*		*
217	Vanillin	*		*		*
	Acids					
A-1	Ethanoic acid				*	*
A-2	Propanoic acid				*	*
A-3	2-Methylpropanoic acid				*	*
A-4	Butanoic acid				*	*
A-5	3-Methylbutanoic acid				*	*
A-6	Pentanoic acid				*	*
A-7	Hexanoic acid				*	*
A-8	Octanoic acid				*	*
A-9	Benzoic acid				*	*
A-10	Tetradecanoic acid				*	*
A-11	Hexadecanoic acid				*	*
A-12	Octadecanoic acid				*	*
	Others					
38	Pyridine	*	*		*	*
46	Acetoin	*				*
123	BHT*	*	*	*	*	*
132	2-Formylpyrrole	*				*
218	Diphenylamine	*				*

BHT (peak 123) is considered to be a contaminant from ether used as solvent.

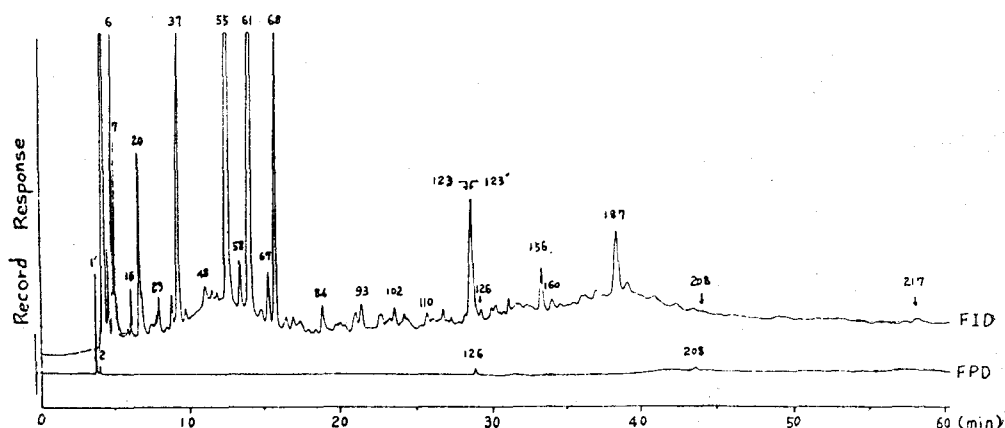


Fig. 2. Gas Chromatogram of the Neutral Fraction obtained from the whole Steam Volatile Concentrate of the Fresh Bellflower Roots

fraction are described individually below.

Volatile components in each fraction

Neutral fraction

Gas chromatogram of the neutral fraction

obtained from the whole steam volatile concentrate of the fresh bellflower roots was given in Figure 2. As shown in Figure 2, about two-hundred components including small peaks as well as large ones on the gas chromatogram

were separated. Characteristically, the large peaks were observed from 5 mins to 16 mins on the gas chromatogram. As a result identified by comparing with the retention times and mass spectra of authentic specimen, these were known to be ethyl acetate (peak 6), ethanol (peak 7), 2,5-dihydrofuran (peak 20), trans-2-hexenal (peak 37), 1-hexanol (peak 55), trans-2-hexenol (peak 61) and 2-furfural (peak 68). 1-Hexenal (peak 21), trans-2-hexenal, 1-hexanol, cis-3-hexenol (peak 58) and trans-2-hexenol among the volatile components identified in the neutral fraction have possessed the green aroma of the bellflower roots. These are well known to be the major green aroma of the fresh tomato fruits. As the results shown in the previous paper⁴⁾, C₆ compounds such as these would be formed through hydrolysis and breakdown of unsaturated fatty acid precursors by enzymatic reaction^{5~12)}. On the other hand, the carbon skeletons of 3-methyl-1-butanal (peak 7') and 3-methyl-1-butanol (peak 34) are similar to that of L-leucine. Probably, these compounds would be biosynthesized by the presence of a specific enzyme catalyzing the conversion of leucine to them^{13~17)}. 2-Furfural, 5-methyl-2-furfural (peak 84), 2,5-dihydrofuran, 2-acetylfuran (peak 78) and

4,7-dimethylbenzofuran (peak 123') are well known to possess characteristic penetrating odor as organoleptic characteristics. The carbon skeletons of these compounds are fundamentally similar to those of pentose such as fructose and/or ascorbic acid. Probably, it would be considered to be formed through thermal degradation of carbohydrates or the oxidation of ascorbic acid during simultaneous steam distillation. β -Ionone (peak 124), dihydroactinidiolide (peak 182) and farnesylacetone (peak 203) are detected as minor components in the neutral fraction. There would be a close relationship between these and carotenoids. These compounds would be formed through the thermal decomposition or the oxidation of carotenoids¹⁸⁾. Farnesol (peak 190) has been used in perfumery to emphasize the odor of sweet floral perfumes, such as lilac and cyclamen. It is also probably formed through the thermal decomposition of carotenoids as mentioned above. Phytol (peak 200) used for preparation of vitamin E and K₁ indicated a weak green-like and sweet floral odor. It would be considered to be biosynthesized through hydrogenation and reduction of geranylgeranyl pyrophosphate during mevalonic acid pathway.

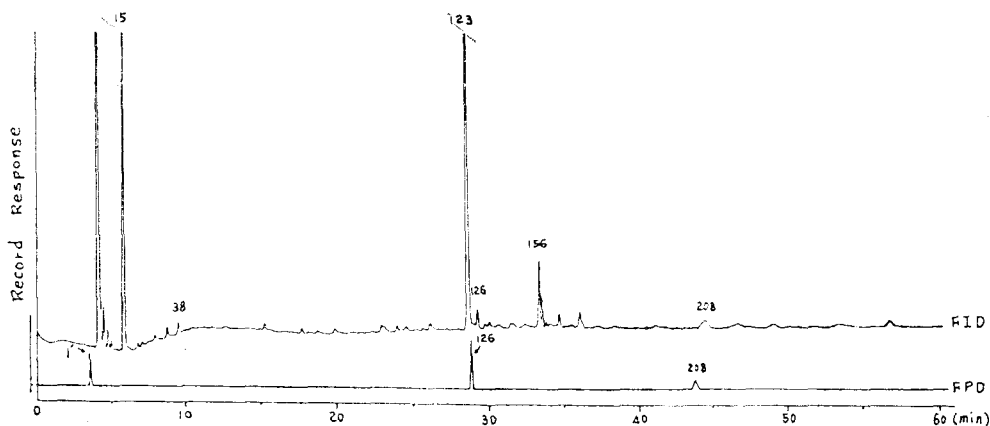


Fig. 3. Gas Chromatogram of the Basic Fraction obtained from the Whole Steam Volatile Concentrate of the Fresh Bellflower Roots

Basic fraction

Gas chromatogram of the basic fraction was given in Figure 3. As shown in Figure 3, all components except for BHT and toluene generally indicated small peaks. In this fraction, pyridine (peak 38), benzothiazole (peak 126) and 2-methylthiobenzothiazole (peak 208) were identified. It is considered that the remainder except for these were contaminated from the neutral fraction during fractionation of the whole steam volatile concentrate. Mass spectrum of pyridine indicating characteristic disagreeable odor and sharp taste showed their ionized fragments at $m/e(\%)$ 79(100), 52(76), 51(40), 26(21), 39(12) and so forth. The molecular ion peak occurred by the removal of a single electron from the molecule is observed as the base peak. Relative intensity of m/e 52 to the base peak indicated 76% and it would be formed by the removal of a nitrile radical from the molecular ion peak. m/e 26 and 39 would be occurred by the removal of C_4H_5 and C_3H_4 cation radicals from the molecular ion peak, respectively. Mass spectral data of benzothiazole indicating odor similar to that of quinoline was as follows: $m/e(\%)$ 135 (100, M^+), 108(24, $M^+ - HCN$), 69 (7, $M^+ - C_3H_3 - HCN$), 63(7, $M^+ - CS - HCN - H$), 82(7, $M^+ -$

$C_4H_4 - H$), 91(6, $M^+ - CS$). Mass spectral data of 2-methylthiobenzothiazole indicating odor similar to that of benzothiazole was as follows: $m/e(\%)$ 181(100, M^+), 148(77, $M^+ - SH$), 108 (39, $M^+ - CH_3S - CN$), 180(28, $M^+ - H$), 69(21, $M^+ - CH_3S - CN - C_3H_3$), 136(18, $M^+ - CS - H$), 45(18, $M^+ - CH_3SH - C_2H_4SH - HCN$), 149(14, $M^+ - S$), 135(13, $M^+ - CH_3S$), 122(10, $M^+ - C_2H_2SH$).

Phenolic fraction

Gas chromatogram of the phenolic fraction was shown in Figure 4. As shown in Table 3, the total of 7 phenolic compounds such as guaiacol (peak 117), phenol (peak 135), o-cresol (peak 135), methyl isoeugenol (peak 160), eugenol (peak 165), 4-vinylguaiacol (peak 173) and 4-vinylphenol (peak 204) were identified as small peaks by means of GC and GC-MS. In the organoleptic test, these indicated odor dissimilar to that of the fresh bellflower roots. Although these individually gave an undesirable odor, it would play an important role for the reproduction of the fresh bellflower root odor. Peak 117 showed the predominant characteristics of the cleavage patterns at $m/e(\%)$ 124(86, M^+), 109(100, $M^+ - CH_3$), 81(49, $M^+ - CH_3CO$), 27(20, $CH_2 = CH$), 39(16,

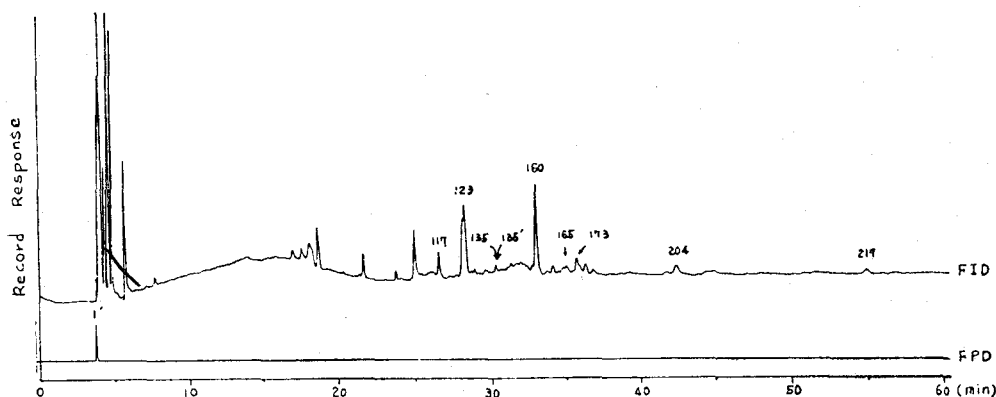


Fig. 4. Gas Chromatogram of the Phenolic Fraction obtained from the Whole Steam Volatile Concentrate of the Fresh Bellflower Roots

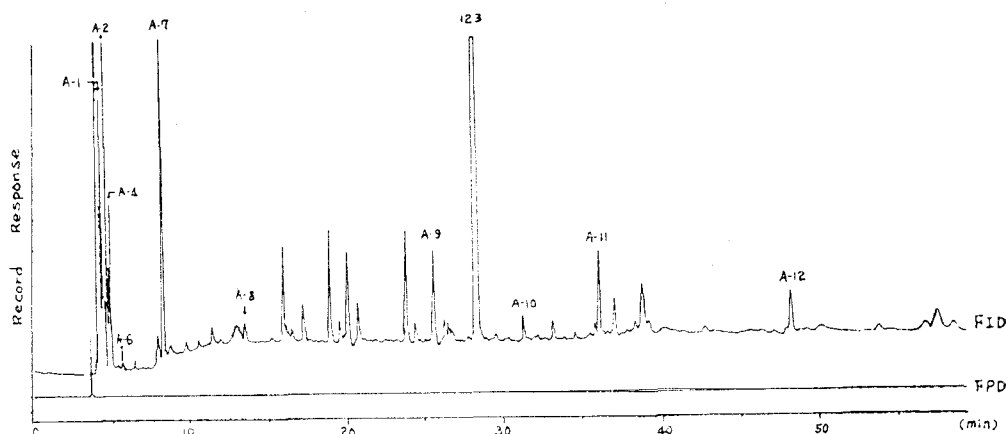


Fig. 5. Gas Chromatogram of the Acidic Fraction obtained from the Whole Steam Volatile Concentrate of the Fresh Bellflower Roots

C_3C_3), 52(14, M^+-CH_3O-CO) and 29(8, $M^+-CH_3O-C_5H_4$). These cleavage patterns due to hetero atom would suggest that this compound is a benzene derivative with a methoxy and a hydroxyl radical in the molecular structure. On the other hand, two phenol compounds, such as 4-vinylguaiaicol and 4-vinylphenol, have been frequently identified in the cooked and/or roasted foods^{19~21}). In our study on the volatile components of the fresh tomato fruits⁴), these two compounds were identified in the volatile concentrate obtained from simultaneous steam distillation, but were not detected in the volatiles of headspace gas. It is well known that they are formed by the thermal decarboxylation of ferulic²²) and p-coumaric acid¹⁹). Probably, there would be a possibility that these compounds were produced by the thermal degradation during GC analysis. Hence, both ferulic and p-coumaric acids would be considered to be the precursors of these compounds.

Acidic fraction

Gas chromatogram of the acidic fraction methylated with diazomethane was given in Figure 5. As shown in Table 3, the total of 12 acids were identified by the techniques

using GC and GC-MS. In GC-MS analysis, the molecular ion peak of a methyl ester of a straight-chain aliphatic acid was clearly observed. The molecular ion peak was generally weak in the range m/e 130 to about 200, but became somewhat more intense beyond this range. In the case of a methyl ester of an aliphatic acid unbranched at the α -carbon, the most characteristic peak due to the familiar McLafferty rearrangement and cleavage one bond removed from the $C=O$ group gave a strong peak at m/e 74. In addition to such tendency, the ion peaks due to elimination of CH_3O from the molecular ion peak and cleavage at each $C-C$ bond gave an important information for the structure elucidation of a methyl ester. On the other hand, methyl benzoate (peak A-9) gave the characteristic cleavage patterns at $m/e(\%)$ 136(32, M^+), 106(8, M^+-CH_3OH), 105(100, M^+-CH_3O), 78(5, C_6H_5+H), 77(65, C_6H_5 or M^+-CH_3COO), 76(4, C_6H_4 or M^+-CH_3COOH), 74(4, $M^+-CH_3OH-HCHO$), 51(36, M^+-CH_3COO C_2H_2) and 39(4, C_3H_3). Mass spectral data of peak A-9 showing the most characteristic strong peak at M^+-31 and M^+-59 would suggest to be a compound having a phenyl and

a CH_3COO group. Hence, this compound was identified to be methyl benzoate, such as a methyl ester of an aromatic acid.

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