

Volatile Compounds Isolated from Edible Korean Chamchwi (*Aster scaber* Thunb)

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The volatile concentrate obtained from the edible Korean chamchwi plant (*Aster scaber* Thunb) by a distillation-extraction system was separated into hydrocarbon and oxygen-containing fractions, and the latter was further separated into eight subfractions by silica gel column chromatography. Gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) were utilized to identify 119 volatile compounds in the fractions. The volatile compounds included 55 hydrocarbons, 37 alcohols, 11 aldehydes, 5 oxides, 4 esters, 4 ketones, 2 acids, and 1 phenol. Myrcene, a monoterpene hydrocarbon, was the most abundant volatile compound identified in chamchwi (18.80%). Chamchwi oil was found to possess a woody or herbaceous aroma following sensory evaluation of each fraction and individual volatile component using a GC-sniff apparatus.

INTRODUCTION

There are thousands of different types of edible plants throughout the world that are utilized in the preparation of meals. Asian cultures primarily use plants as raw materials to make vegetable soups and seasoned greens. With careful selection, plants can provide an adequate balance of the proteins, carbohydrates, fats, vitamins, and mineral acids required for good health. Plant materials are both volatile and nonvolatile and affect odor and flavor profiles as well as sensory impact. The volatile constituents give a plant its distinctive odor, whereas the nonvolatile compounds are either inert or may be implicated in a diverse number of physiological effects when consumed. They can also serve in many cases as precursors for the formation of volatile flavor compounds. The intensity of the aroma and types of physiological effect show wide variations between plant families and species.

Chamchwi (*Aster scaber* Thunb) is a perennial herb of the Compositae family and grows wild in the dry mountain regions of Korea. Young, edible chamchwi is gathered in early spring and used in the preparation of traditional Korean dishes. The greens are seasoned to make salads or are boiled together with soybean paste and anchovy extracts in the preparation of soups.

The nonvolatile components of *A. scaber* Thunb have recently become the subject of research in several laboratories throughout the world (Ham et al., 1991; Nagao et al., 1991a,b; Nagao and Okabe, 1992; Payne-Whal and Kleiman, 1981; Tada et al., 1974; Zdoroveva and Gorovoi, 1972). However, no attempts have previously been made to characterize the volatile constituents of this plant. The object of the present investigation, then, was to identify and characterize the volatile compounds isolated from *A. scaber* Thunb by gas chromatography (GC), gas chromatography/mass spectrometry (GC/MS), and GC-sniff sensory analysis.

EXPERIMENTAL PROCEDURES

Materials. Young leaves of chamchwi, grown in the Cheon-seong Mountains (Yangsang, Korea), were collected in April 1991. Diethyl ether, *n*-pentane, and anhydrous sodium sulfate were

obtained from Sigma Chemical Co. (St. Louis, MO). All authentic chemicals were purchased from reliable commercial sources.

Sample Preparation. The volatile concentrate was prepared with a modified Likens-Nickerson distillation-extraction system (SDE) at atmospheric pressure. A sample flask containing 500 g of freshly crushed leaves and 1.5 L of distilled water was attached to the lower right joint of the SDE, and a small flask containing 200 mL of redistilled diethyl ether, the extracting solvent, was attached to its left joint. The sample flask was heated to 100 °C by an electric heating mantle, and the extracting solvent flask was kept at 38 °C by a water bath. The SDE was allowed to proceed for 2 h, and the extracting solvent was collected. The ether extract was dried over anhydrous sodium sulfate for 12 h. After removal of sodium sulfate, the extract was concentrated to 1 mL by fractional distillation at 38 °C and atmospheric pressure. The above procedure was repeated 40 times utilizing all of the leaves (20 kg), and the extracts were subsequently combined.

Fractionation of the Volatile Extract. The whole volatile concentrate was fractionated into hydrocarbon and oxygen-containing compound fractions by a silica gel column (1 × 46 cm). The hydrocarbon and oxygen-containing fractions were eluted with *n*-pentane (500 mL) and redistilled diethyl ether (500 mL), respectively. Both extracts were concentrated to 0.5 mL, and the diethyl ether eluate was further separated by a similar silica gel column into eight subfractions by gradient elution of *n*-pentane and diethyl ether. *n*-Pentane content in each elution was 98%, 97%, 95%, 93%, 90%, 80%, 50%, and 0%, respectively. The resulting fractions were similarly concentrated to 0.5 mL by fractional distillation. The concentrated extracts were then placed in vials and stored under argon at -4 °C until analysis.

Instrumental Analysis. A Hewlett-Packard (HP) Model 5890A gas chromatograph equipped with a flame ionization detector (FID) was used for routine analysis. Three different types of fused silica WCOT capillary columns (50 m × 0.25 mm i.d.) coated with HP-101 (dimethylsilicone), HP-20M (Carbowax), and HP-FFAP (free fatty acid phase) stationary phases were used. The GC oven temperature of the HP-101 was programmed from 70 to 200 °C, that of the HP-20M from 60 to 190 °C, and that of the HP-FFAP from 60 to 210 °C, all at a rate of 3 °C/min. The injector and detector temperatures were both kept at 230 °C. Nitrogen (0.6 mL/min) was used as the carrier gas, and the split ratio was set to 1:100.

A HP Model 5890A GC interfaced to a HP 5970 mass spectrometer was used for MS identification of the GC components using the same columns and oven conditions listed above. Helium (1.0 mL/min) was used as the carrier gas. The mass spectra were obtained by electron impact ionization at 70 eV and an ion source temperature of 250 °C. The spectral data were recorded on a HP 59970A computer data system. All compound identifications were made by comparing the Kovats retention index (Kovats, 1965) and MS fragmentations to those of authentic

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Table I. Yield and Odor Characteristics of the Volatile Flavor Concentrate of Chamchwi and Subsequent Fractions

fraction	yield, ^a g	odor ^b	compounds
volatile concentrate	0.385	chamchwi-like	all volatile components
hydrocarbon fraction	0.359	woody or herbaceous	hydrocarbons
oxygen-containing compound fraction	0.026	chamchwi-like	oxygen-containing compounds
OCCF-1	0.006	sweet-floral	aldehydes, esters
OCCF-2	0.001	herbaceous	aldehydes, oxides
OCCF-3	0.003	sweet-floral	mono- and sesquiterpene alcohols
OCCF-4	0.004	sweet-floral	mono- and sesquiterpene alcohols
OCCF-5	0.009	woody or herbaceous	alkenols, monoterpene alcohols, aldehydes
OCCF-6	0.002	woody or herbaceous	alkenols, aromatic aldehydes, monoterpene alcohols
OCCF-7	0.001	floral	acids, aromatic alcohols
OCCF-8	0.0003	medicinal	not identified

^a Yield was obtained from 20 kg of chamchwi. The yield for each fraction was found by subtracting the solvent peak area from total peak area and dividing by total peak area to obtain a ratio. This value was then multiplied by the weight (g) of the extract.

Table II. Volatile Compounds Identified in the Extract of Edible Korean Chamchwi (*A. scaber* Thunb)

compound	RI ^a			GC peak area, %			
	HP-101	HP-20M	HP-FFAP	volatile concentrate	hydrocarbon fraction	oxygen-containing compound fraction	subsequent OCCF fraction
ethanal	418	714	718	0.38		0.69	1
ethanol	485	929	936	0.52		0.49	4, 5
ethyl formate	530	848	837	0.43		0.96	1
ethyl acetate	602	907	902	0.65		1.46	1
3-methylbutanal	641	929	936	0.06		0.06	1
pentanal	687	986	1004	0.09		0.10	1
acetic acid	710	1404	1471	0.09		0.13	7
3-methyl-1-butanol	736	1182	1213	0.12		0.12	5, 6
toluene	782	1042	1070	0.04	0.06		
hexanal	782	1075	1107	0.09		0.12	1
octane	800	800	800	b	b		
(<i>E</i>)-2-hexenal	836	1209	1250	0.34		0.28	1, 2
(<i>Z</i>)-3-hexenal	860	1357	1400	1.34		1.65	5, 6
1-hexanol	866	1325	1361	0.40		0.63	5, 6
(<i>E</i>)-2-hexenol	870	1377	1419	0.32		0.47	5, 6
heptanal	885	1174	1208	0.04		0.10	1
nonane	900	900	900	b	0.02		
α -thujene	930	1021	1031	0.12	0.19		
α -pinene	941	1034	1045	2.72	2.85		
camphene	957	1075	1097	0.15	0.14		
benzaldehyde	965	1500	1586	0.09		0.18	1
6-methyl-5-hepten-2-one	973	1319	1365	0.03		0.08	2, 3
sabinene	977	1123	1147	1.92	1.16		
1-octen-3-ol	981	1423	1458	0.04		0.10	4
β -pinene	985	1114	1138	2.31	2.56		
myrcene	990	1161	1180	18.80	22.41		
3-octanol	995	1368	1400	b		0.13	4
decane	1000	1000	1000	0.03	b		
α -phellandrene	1009	1166	1194	0.45	0.43		
3-carene	1017	1148	1180	0.21	0.20		
α -terpinene	1020	1178	1208	0.11	0.18		
<i>p</i> -cymene	1025	1261	1303	0.78	0.92		
limonene	1035	1198	1229	5.68	6.61		
1,8-cineole	1035	1209	1243	0.18		0.26	4
(<i>Z</i>)-ocimene	1035	1225	1252	0.40	0.59		
β -phellandrene	1035	1209	1243	10.61	13.33		
(<i>E</i>)-ocimene	1043	1242	1270	1.99	2.50		
phenylacetaldehyde	1047	1615	1706	0.32		1.02	4, 5, 6
γ -terpinene	1058	1238	1274	0.20	0.49		
1-octanol	1071	1530	1567	0.08		0.42	5
(<i>Z</i>)-linalool oxide (furanoid)	1071	1420	1471	0.11		0.16	5
benzyl alcohol	1089	1837	1927	0.11		0.36	6
terpinolene	1089	1275	1315	2.09	2.69		
(<i>E</i>)-linalool oxide (furanoid)	1089	1449	1449	0.06		0.33	5
linalool	1100	1522	1558	2.59		5.78	3
undecane	1100	1100	1100	b	0.18		
perillen	1109	1295	1331	0.03	0.05		
α -fenchyl alcohol	1123	1557	1615	0.03		0.05	3
(<i>E</i>)- <i>p</i> -2-menthen-1-ol	1128	1538	1594	0.20		0.45	4
2-phenylethanol	1152	1873	1965	0.42		1.51	6
(<i>E</i>)-pinocarveol	1152	1832	1706	0.11		0.59	4, 5
lavandulol	1169	1677	1781	0.12		0.40	3, 4, 5
borneol	1180	1677	1747	0.14		0.66	3, 4, 5
terpinen-4-ol	1182	1579	1642	1.08		3.36	3, 4, 5, 6
neoisomenthol	1182	1646	1747	0.33		1.31	3, 4, 5
α -terpineol	1197	1669	1732	0.94		1.92	5, 6

Table II (Continued)

compound	RI ^a			GC peak area, %			
	HP-101	HP-20M	HP-FFAP	volatile concentrate	hydrocarbon fraction	oxygen-containing compound fraction	subsequent OCCF fraction
naphthalene	1197	1718	1825	0.06	0.04		
dodecane	1200	1200	1200	b	0.14		
<i>p</i> -cymen-8-ol	1200	1814	1887	0.35		1.29	3, 4, 5
(<i>Z</i>)-piperitol	1203	1651	1698	0.27		0.16	4, 5
(<i>E</i>)-piperitol	1216	1653	1719	0.13		0.45	4, 5
citronellol	1229	1737	1781	0.04		0.11	5
neral	1231	1658	1724	0.04		0.12	3, 4, 5
nerol	1234	1770	1825	0.12		0.28	4, 5, 6
isogeraniol	1237	1782	1849	0.16		0.39	4, 5
cuminic aldehyde	1241	1759	1854	0.06		0.22	4
geraniol	1258	1814	1867	0.32		1.18	4, 5, 6
geranial	1258	1708	1776	0.17		0.62	3, 4, 5
methyl (<i>E</i>)-geranate	1284	1566	1620	0.19		0.52	1
tridecane	1300	1300	1300	0.06	0.09		
cuminic alcohol	1322	2058	2144	0.13		0.41	4, 5, 6
eugenol	1381	2122	2219	0.17		0.33	3
α -copaene	1389	1488	1536	0.13	0.24		
β -bourbonene	1400	1585	1633	1.86	2.88		
tetradecane	1400	1400	1400	b	0.06		
isocaryophyllene	1421	1570	1627	0.52	0.42		
β -caryophyllene	1438	1594	1657	5.43	10.10		
(<i>E</i>)- β -farnesene	1451	1658	1711	0.60	0.14		
aromadendrene	1458	1600	1666	0.36	0.30		
α -humulene	1472	1663	1732	1.83	1.26		
alloaromadendrene	1479	1639	1706	0.29	0.48		
β -ionone	1484	1912	2001	0.36		1.13	2, 3
γ -muurolene	1488	1681	1741	1.17	2.53		
germacrene D	1500	1705	1772	7.29	9.85		
pentadecane	1500	1500	1500	b	0.07		
α -muurolene	1506	1714	1788	0.41	2.57		
β -selinene ^c	1511	1711	1781	0.46	0.86		
β -bisabolene ^c	1513	1714	1788	0.52	2.25		
(<i>E,E</i>)- α -farnesene	1518	1725	1801	0.91	1.89		
γ -cadinene	1526	1752	1819	0.33	0.82		
δ -cadinene	1531	1749	1808	1.42	3.14		
α -curcumene ^c	1536	1773	1841	0.09	0.11		
β -sesquiphellandrene	1543	1782	1849	0.09	0.15		
calamenene	1549	1927	1930	0.12	0.19		
(<i>E</i>)-nerolidol	1564	2009	2055	0.15		0.64	3, 4, 5
caryophyllene oxide	1600	1962	2068	0.39		1.52	2, 3, 4
hexadecane	1600	1600	1600	b	0.02		
β -bisabolol	1603	2021	2106	0.40		1.68	3
humulene oxide	1625	2015	2122	0.11		0.69	2
β -caryophyllene alcohol ^c	1625	2003	2122	0.25		1.14	4
3-oxo- β -ionone	1648	2106	2195	0.13		0.77	2, 3
δ -cadinol	1657	2134	2219	0.38		1.33	4, 5
<i>T</i> -muurolol	1659	2148	2234	0.81		3.36	4, 5, 6
α -cadinol	1674	2191	2282	1.36		5.97	5, 6
α -caryophyllene alcohol ^c	1679	2209	2310	0.48		2.10	5
heptadecane	1700	1700	1700	0.07	b		
2-hexadecanone	1781	2092	2150	b		0.13	2
octadecane	1800	1800	1800	b	b		
α -calacorene ^c	1842			0.02	0.17		
nonadecane	1900	1900	1900	b	b		
palmitic acid	2000	2860	2940	b		b	7
eicosane	2000	2000	2000	b	b		
5-hydroxycalamenene	2068	2325	2437	0.18		1.31	4
heneicosane	2100	2100	2100	b	b		
phytol	2111	2571	2593	0.08		0.60	4, 5
docosane	2200	2200	2200	b	b		
tricosane	2300	2300	2300	b	b		
known compounds				91.76	98.33	54.78	
unknown compounds				8.24	1.67	45.22	

^a Kovats index calculated on HP-101, HP-20M, and HP-FFAP capillary columns. ^b Peak area percent less than 0.02. ^c Tentatively identified.

samples. Tentative identifications were made in cases for which an authentic reference standard was not available and identification could only be made on the basis of MS data.

Sensory Evaluation. An aliquot of ether solution from each fraction was adsorbed on a filter paper and air-dried to remove the solvent. The whole volatile concentrate and its subsequent fractions were then evaluated by 10 members of the laboratory.

Sensory evaluation of the individual volatile constituents emitted at each peak on the gas chromatogram was accomplished by sniffing at the exit port of a Gasukuro Kygyo Model 350 GC equipped with an effluent splitter and an FID. The GC capillary columns and oven conditions were the same as stated above. Carrier gas was kept at a flow rate of 3.48 mL/min with a split ratio of 1:1.5.

Table III. Organoleptic Characteristics of Each Volatile Component Identified from Edible Korean Chamchwi (*A. scaber* Thunb)

sensory note	compounds ^a
herbaceous	3-methylbutanal, pentanal, 1-octen-3-ol, 3-octanol, (<i>Z</i>)-ocimene, (<i>E</i>)-ocimene, (<i>E</i>)- <i>p</i> -2-menthen-1-ol, neoisomenthol, (<i>Z</i>)-piperitol, (<i>E</i>)-piperitol, cuminic aldehyde, cuminic alcohol, β -bourbonene, γ -muurolene, β -selinene, β -bisabolene, γ -cadinene, δ -cadinene, α -curcumene, calamenene, caryophyllene oxide, humulene oxide, β -bisabolol, β -caryophyllene alcohol, α -caryophyllene alcohol, δ -cadinol, <i>T</i> -muurolol, α -cadinol, 5-hydroxycalamenene, phytol
woody	α -thujene, α -pinene, benzaldehyde, 6-methyl-5-hepten-2-one, sabinene, β -pinene, myrcene, terpinolene, perillen, α -copaene, isocaryophyllene, β -caryophyllene, (<i>E</i>)- β -farnesene, aromadendrene, α -humulene, alloaromadendrene, β -ionone, germacrene D, α -muurolene, (<i>E,E</i>)- α -farnesene, β -sesquiphellandrene, 3-oxo- β -ionone, a-calacorene
floral	phenylacetaldehyde, 1-octanol, (<i>Z</i>)-linalool oxide, (<i>E</i>)-linalool oxide, linalool, 2-phenylethanol, (<i>E</i>)-pinocarveol, lavandulol, α -terpineol, <i>p</i> -cymen-8-ol, citronellol, nerol, isogeraniol, geraniol, methyl (<i>E</i>)-geranate, eugenol, (<i>E</i>)-nerolidol
fruity	heptanal, α -phellandrene, α -terpinene, <i>p</i> -cymene, limonene, β -phellandrene, γ -terpinene, benzyl alcohol, terpinen-4-ol, neral, geranial, 2-hexadecanone
fusel-like	octane, nonane, decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, heptadecane, octadecane, nonadecane
pungent	ethanal, ethanol, ethyl formate, ethyl acetate, acetic acid, 3-methyl-1-butanol, toluene
leafy	hexanal, (<i>E</i>)-2-hexenal, (<i>Z</i>)-3-hexenol, 1-hexanol, (<i>E</i>)-2-hexenol
camphoraceous	camphene, 1,8-cineole, α -phenyl alcohol, borneol
odorless	eicosane, heneicosane, docosane, tricosane
oily	palmitic acid
medicinal	naphthalene

^a Overlapping GC peaks were evaluated by injection of authentic compounds.

RESULTS AND DISCUSSION

All sensory panelists noted that the aroma of the volatile concentrate was very similar to that of fresh chamchwi, having an oily herbaceous odor without off-flavors. The yields and odor characteristics of the whole volatile concentrate and its fractions/subfractions are summarized in Table I. The odor of the hydrocarbon fraction (HF) and the oxygen-containing compound fraction (OCCF) was less pleasant than that of the original volatile flavor concentrate. However, the oxygen-containing fraction retained mild chamchwi-like odor and was hence considered to be more important in characterizing chamchwi aroma. The oxygen-containing fraction, then, was further separated into eight subfractions by silica gel column chromatography, and each subfraction was subjected to sensory evaluation. Among the subfractions, some carried floral notes, while others bore more woody and herbaceous odors.

The approximate yield of the volatile flavor concentrate obtained from 20 kg of chamchwi was 0.385 g (0.002%). One hundred nineteen compounds of approximately 200 peaks on the gas chromatogram were identified in the volatile concentrate. These volatile components are listed in Table II by elution order from a HP-101 capillary column. Table II was prepared by the method reported by Flath et al. (1983). Some compounds were not positively identified because of the lack of authentic chemical, so Kovats index and mass spectra could not be compared. The compounds identified included 55 hydrocarbons (72.68%), 37 alcohols (14.88%), 11 aldehydes (1.68%), 5 oxides (0.85%), 4 esters (1.27%), 4 ketones (0.52%), 2 acids (0.09%), and 1 phenol (0.17%). Percentage contributions of the various compound classes were calculated by the GC peak area represented in the whole volatile concentrate chromatogram. Hydrocarbons comprised the majority of the components identified and included 16 alkanes (0.16%), 20 sesquiterpene hydrocarbons (23.85%), 17 monoterpene hydrocarbons (48.57%), and 2 aromatic

hydrocarbons (0.10%). The major component of the monoterpene hydrocarbons was myrcene (18.80%), and that of the sesquiterpene hydrocarbons was germacrene D (7.29%).

Alcohols, the next most predominant class of volatiles identified, were composed of 5 alkanols (1.12%), 3 alkenols (1.70%), 3 aromatic alcohols (0.66%), 16 monoterpene alcohols (6.93%), 9 sesquiterpene alcohols (4.39%), and 1 diterpene alcohol (0.08%). Phytol, which is the monounsaturated diterpene alcohol incorporated in the chlorophyll molecule, has been thought to be formed by degradation of chlorophyll in plants (Mann, 1987), and this is similarly proposed for chamchwi.

Aldehydes were composed of six alkanals (1.00%), three aromatic aldehydes (0.47%), and two monoterpene aldehydes (0.21%). Hexanal and (*E*)-2-hexenal were probably formed from the enzyme-induced oxidative breakdown of unsaturated fatty acids including linoleic and linoleic acids (Pyne and Wick, 1965; Kazeniak and Hall, 1970; Chung and Lee, 1991). 6-Methyl-5-hepten-2-one, β -ionone, and 3-oxo- β -ionone among the four ketones identified in chamchwi may arise from the oxidative breakdown of lycopene and β -carotene pigments in the leaves (Stevens, 1970; Marais et al., 1990).

The organoleptic characteristics of each volatile component by GC-sniff evaluation are listed in Table III. The results of sniffing the effluents at the exit port of the gas chromatograph revealed the absence of an individual volatile compound solely possessing a characteristic chamchwi-like aroma. Thirty-one of the 119 volatile components identified had a somewhat herbaceous aroma and were composed of 16 alcohols, 10 hydrocarbons, 3 aldehydes, and 2 oxides. Twenty-three compounds possessed a somewhat woody aroma and included 19 hydrocarbons, 3 ketones, and 1 aldehyde. Therefore, it is likely that a mixture of the above volatiles invokes the characteristic chamchwi aroma. These herbaceous and woody aroma compounds would deserve the most attention in an

attempt to reconstruct chamchwi essential oil. Furthermore, several unidentified volatile flavor components probably contribute and may play an important role in the aroma of chamchwi essential oil.

The most abundant monoterpenes isolated in chamchwi are myrcene (18.80%), β -phellandrene (10.61%), and limonene (5.68%). The biosynthesis of the acyclic monoterpene myrcene undoubtedly arises from the chemical modification of either geranyl pyrophosphate (GPP) or neryl pyrophosphate (NPP). The cyclic monoterpenes β -phellandrene and limonene, however, are probably only derived from NPP, for the reason that it is not stereochemically possible for GPP to cyclize directly (Banthorpe and Charlwood, 1972). The major sesquiterpenes isolated are germacrene D (7.29%), β -caryophyllene (5.43%), and β -bourbonene (1.86%), from which they are thought to be derived from the cyclization of various isomers of farnesyl pyrophosphate (FPP) (Banthorpe and Charlwood, 1972).

Characterization of the volatile components of Korean chamchwi has also provided significant insight into its practical medicinal uses. For example, myrcene, the most abundant compound identified, has been shown to possess potent analgesic activity in rats (Lorenzetti et al., 1991) and at therapeutic doses shows no toxic effects (Paumgarten et al., 1990). Limonene, another major monoterpene identified in this study, has previously been shown to inhibit rat mammary carcinogenesis (Russin et al., 1989). Recently, *d*-limonene has been shown to induce the activity of the detoxifying enzyme, GST, indicating its promise as a potential anticarcinogenic agent (Zheng et al., 1993).

The isolation and identification of volatile aroma compounds from the edible Korean chamchwi plant (*A. scaber* Thunb) is a novel endeavor. Characterizing the volatile components and sensory characteristics of chamchwi essential oil is necessary to begin to study and understand its role in food flavor and its potential applications in the fields of medicine and toxicology. However, to fully understand the significant volatiles implicated in chamchwi oil aroma and chemistry, further characterization of unidentified oxygen-containing compounds is necessary and is currently in progress.

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