

## RESEARCH REPORT

# Essential Oil Composition of *Origanum ramonense* Danin Leaves from Israel

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## Abstract

Essential oils of *Origanum ramonense* Danin leaves were obtained using two methods: steam-distillation followed by dichloromethane extraction (SDR), and simultaneous purging and extraction (SPE). Volatile constituents of the oils were isolated and identified by GC and GC/MS. A total of 132 compounds were identified using both methods. Among the more than 90 compounds identified in the oil obtained by SDR, 42 were alcohols which composed 79.5% of total GC peak area. The major volatile constituents of this oil were  $\alpha$ -terpineol (41.53%), terpinen-4-ol (16.80%), *cis*-sabinene hydrate (13.17%), eugenol (3.61%), and  $\gamma$ -terpinene (3.40%). Among 90 constituents identified in oil obtained by SPE, 28 were hydrocarbons which composed 55.70% of total GC peak area. The major constituents of oil obtained by SPE were 1,8-cineol (17.46%),  $\gamma$ -terpinene (14.69%), terpinen-4-ol (13.88%),  $\alpha$ -terpinene (9.37%) and  $\alpha$ -terpineol (6.83%).

## Key Word Index

*Origanum ramonense*, Labiatae, essential oil composition, headspace composition,  $\alpha$ -terpineol, terpinen-4-ol, *cis*-sabinene hydrate, 1,8-cineole,  $\gamma$ -terpinene, caryophyllene derivatives

## Introduction

*Origanum* is a herbaceous plant which grows wild in sunny spots throughout all continents. A number of botanical sources are classified under the name "origans" including *Origanum ramonense*

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Table I. Composition of *Origanum ramonense* oil from Israel

Compound	I <sup>b</sup>	GC peak area% <sup>a</sup>		Compound	I <sup>b</sup>	GC peak area% <sup>a</sup>	
		SDR <sup>c</sup>	SPE <sup>d</sup>			SDR <sup>c</sup>	SPE <sup>d</sup>
<b>Alcohols</b>				<b>Aldehydes</b>			
2-methyl-3-buten-2-ol	1037	e	-	acetaldehyde		e	-
isobutanol	1091	e	-	hexanal	1080	-	0.02
butanol	1143	e	-	(Z)-3-hexenal	1137	e	-
1-penten-3-ol	1158	e	e	(E)-2-hexenal	1216	0.01	0.04
pentanol	1251	e	-	nonanal	1390	-	e
4-pentenol*	1303	e	-	(E,Z)-2,4-heptadienal	1460	e	-
(Z)-2-pentenol	1321	0.01	-	decanal	1495	-	e
hexanol	1355	e	e	benzaldehyde	1514	e	0.01
(E)-3-hexenol	1365	e	-	myrtenal	1621	-	e
(Z)-3-hexenol	1386	0.10	-	<i>Total GC peak area% of aldehydes</i>		0.01	0.07
3-octanol	1397	e	0.01	<b>Ketones</b>			
(E)-2-hexenol	1408	0.02	0.01	acetone	815	0.01	0.02
1-octen-3-ol	1453	0.08	0.11	2-butanone*	913	-	0.01
<i>trans</i> -sabinene hydrate	1470	2.78	-	3-methylbutan-2-one	938	-	0.01
2-ethylhexanol	1492	e	0.02	2,3-butanedione	972	e	0.02
linalool	1549	-	0.30	3-octanone	1253	e	0.01
<i>cis</i> -sabinene hydrate	1555	13.17	-	6-methyl-5-hepten-2-one	1336	-	e
<i>p</i> -menth-2-en-1-ol* (tentative)	1567	1.26	e	chrysanthenone	1501	0.01	0.02
$\alpha$ -lenchol	1582	-	e	camphor	1509	0.13	0.10
terpinen-4-ol	1605	16.80	13.88	pinocarvone	1560	-	0.07
isoborneol	1665	-	0.01	piperitone	1728	0.02	-
piperitol*	1673	-	e	carvone	1734	e	-
<i>trans</i> -verbenol	1679	0.59	-	isopiperitenone	1831	0.02	-
<i>p</i> -menth-1,8-dien-4-ol	1686	0.57	0.09	$\beta$ -ionone	1932	e	-
$\alpha$ -terpineol	1709	41.53	6.83	5,8-epoxy- $\beta$ -ionone	1983	e	-
borneol	1709	0.96	1.57	caryophylla-2(12),6(13)-dien-5-one	2033	0.02	e
isopiperitenol*	1750	0.52	e	caryophylla-2(12),6-dien-5-one	2075	0.01	-
isopiperitenol isomer*	1754	0.02	e	<i>Total GC peak area % of ketones</i>		0.20	0.25
pinocarveol*	1787	0.03	-	<b>Hydrocarbons</b>			
myrtenol	1791	0.10	0.02	benzene	939	-	e
<i>p</i> -menth-1(7),8-dien-2-ol*	1796	0.05	0.01	tricyclene	1009	-	0.20
nerol	1802	0.02	-	$\alpha$ -pinene	1021	e	2.91
<i>p</i> -cymen-8-ol	1846	0.22	0.03	$\alpha$ -thujene	1025	0.02	0.54
<i>trans</i> -myrtenol	1859	0.06	-	toluene	1037	e	0.03
<i>cis</i> -carveol	1863	0.02	-	camphene	1063	e	4.87
<i>cis</i> -myrtenol	1868	0.01	-	$\beta$ -pinene	1107	e	1.15
benzyl alcohol	1871	0.02	-	sabinene	1119	0.03	0.04
<i>p</i> -menth-1(7),8-dien-2-ol isomer*	1885	0.06	0.01	<i>p</i> -menthene	1133	-	0.01
2-phenylethyl alcohol	1905	0.02	-	<i>m</i> -xylene	1137	-	e
2,6-di- <i>tert</i> -butyl- <i>p</i> -cresol	1906	-	e	myrcene	1163	0.53	4.73
<i>p</i> -menth-1-en-9-ol*	1932	e	-	$\alpha$ -phellandrene	1167	-	e
<i>p</i> -menth-1-en-9-ol diastereomer*	1935	0.01	-	$\alpha$ -terpinene	1181	2.89	9.37
perillyl alcohol	2000	0.02	-	limonene	1198	0.08	4.38
cinnamyl alcohol	2093	0.03	-	$\beta$ -phellandrene	1212	1.13	4.24
caryophylla-2(12),6(13)-dien-5-ol	2286	0.42	e	<i>p</i> -ethyltoluene	1221	-	e
caryophylla-2(12),6-dien-5-ol*	2322	0.06	e	(Z)- $\beta$ -ocimene	1234	-	e
caryophylla-2(12),6-dien-5-ol isomer*	2327	0.01	-	$\gamma$ -terpinene	1247	3.40	14.69
<i>Total GC peak area% of alcohols</i>		79.54	22.88	(E)- $\beta$ -ocimene	1251	-	0.01

Table I. Continued

Compound	GC peak area% <sup>a</sup>			Compound	GC peak area% <sup>a</sup>		
	l <sup>b</sup>	SDR <sup>c</sup>	SPE <sup>d</sup>		l <sup>b</sup>	SDR <sup>c</sup>	SPE <sup>d</sup>
<i>p</i> -cymene	1267	0.04	4.90	<b>Miscellaneous</b>			
1,2,4-trimethylbenzene	1277	-	e	2,3-dehydro-1,8-cineole	1191	e	e
terpinolene	1282	0.83	2.60	1,8-cineole	1212	2.15	17.46
tetradecane	1400	-	e	terpinen-4-yl ethyl ether <sup>f</sup>	1384	-	0.02
dehydro- <i>p</i> -cymene	1432	0.01	0.05	ethyleneglycol monobutyl ether <sup>f</sup>	1403	0.01	-
$\beta$ -caryophyllene	1593	-	0.95	3-(4-methyl-3-pentenyl)furan			
$\alpha$ -humulene	1661	-	0.03	(perillene)	1417	-	e
pentacosane	2500	-	e	<i>cis</i> -linalool oxide (furanoid)	1442	0.02	0.01
heptacosane	2700	-	0.01	$\alpha$ -terpinyl ethyl ether	1444	-	0.02
<b>Total GC peak area% of hydrocarbones</b>		8.95	55.70	<i>trans</i> -linalool oxide (furanoid)	1472	0.03	0.01
<b>Esters</b>				benzothiazole	1943	e	-
ethyl acetate	902	-	e	caryophyllene oxide	1975	0.58	0.01
ethyl butyrate	1033	-	0.02	phenol	1997	e	-
1-octen-3-yl acetate	1379	-	0.24	methyl eugenol	2006	e	-
linalyl acetate	1555	-	e	<i>p</i> -cresol	2073	e	e
bornyl formate	1589	-	0.03	eugenol	2158	3.61	-
bornyl acetate	1581	0.31	1.15	thymol	2176	e	-
terpinen-4-yl acetate	1616	0.06	0.19	2-methoxy-4-vinylphenol			
pinocarvyl acetate <sup>*</sup>	1645	-	0.01	(4-vinylgualacol)	2178	e	e
myrtenyl acetate	1685	-	e	carvacrol	2202	0.07	e
$\alpha$ -terpinyl acetate	1692	-	0.04	<b>Total GC peak area% of miscellaneous</b>		6.47	17.53
$\alpha$ -terpinyl formate	1716	-	0.08				
methyl salicylate	1767	0.02	0.02				
diethyl phthalate <sup>f</sup>	2351	e	-				
diisobutyl phthalate <sup>f</sup>	2518	e	-				
dibutyl phthalate <sup>f</sup>	2663	e	0.02				
<b>Total GC peak area% of esters</b>		0.39	1.79				

<sup>a</sup> Solvent is excluded; <sup>b</sup> Kovats Index on DB-Wax; <sup>c</sup> Volatiles obtained by steam distillation under reduced pressure; <sup>d</sup> Volatiles obtained by simultaneous purging and extraction; <sup>e</sup> GC peak area% less than 0.01; <sup>f</sup> possibly an artifact; \* correct isomer not identified

Danin. It reaches 30-80 cm in height and exhibits an oblique rhizome, erect flower-bearing stalks (1). The most common herb of *Origanum* species is *O. vulgare* L., the source of the essential oil known as oil of wild marjoram. There are also several other *Origanum* species, such as *O. syriacum* L. (syn. *Majorana syriacus* L. Kostelerzky) and *O. syriacum* L. var. *syriacum* (syn. *O. maru* L.) which are native in ~~West~~ <sup>East</sup> Mediterranean countries such as Israel. *O. ramonense* Danin is endemic to Israel.

Commercial *Origanum* oil is obtained from the dried leaves of *Thymus* and *Origanum* species using steam distillation. The oil is used in perfumery for its powerful refreshing notes and its spicy-herbaceous aroma. The composition of oils of various *Origanum* species has been investigated. It was postulated that the oil exists in two forms, one a predominant *cis*-sabinene hydrate (*cis*-thujanol-4) and the other a predominant terpinen-4-ol (2). Later, it was suggested that there is no natural terpinene-4-ol type in the oil (3). In the present study, the oil obtained from leaves of *O. ramonense* was analyzed using GC and GC/MS.

## Experimental

**Plant Material:** The leaves of *Origanum ramonense* were collected at the Negev Highlands, Nahal Elot, Israel on February 14, 1995. The plant was found at 900 m elevation in crevices of the smooth-faced limestone outcrop. The leaves were sorted on white paper to remove stem, bark, soil, and other non-leaf materials. They were then spread out on a large sheet of paper to dry at room temperature, 25°C for 24 h. After air drying, the leaves were ground using a mortar and pestle.

**Reference Compounds:** Authentic compounds were purchased from Aldrich Chemical Co. (Milwaukee, WI), Tokyo Kasei Organic Chemicals (Tokyo, Japan), Wako Pure Chemical Industries Ltd. (Osaka, Japan), and Fluka Chemical Co. (Ronkonkoma, NY). Chemicals which were not commercially available were synthesized according to the published methods. *cis*-Sabinene hydrate was synthesized by the method of Fischer et al. (4). Caryophylla-2(12),6(13)-dien-5-one and caryophylla-2(12),6-dien-5-one were synthesized by the method reported by Kaiser et al. (5) and their alcohols, caryophylla-2(12),6(13)-dien-5-ol and caryophylla-2(12),6-dien-5-ol were synthesized by the method of Kasano et al. (6).

**Isolation of Essential Oil by Steam Distillation under Reduced Pressure (SDR):** The powdered dried leaf of *O. ramonense* (100 g) was mixed with 2 L distilled water and 150 mL saturated sodium chloride solution in a 3 L round bottom flask. The solution was hydrodistilled at 33°C for 30 min under reduced pressure (33 mmHg). The distillate (900 mL) was extracted with 90 mL dichloromethane using a liquid-liquid continuous extractor for 4 h. After the extract was dried over anhydrous sodium sulfate, the solvent was removed by a distillation with a Vigreux column. The distillation was stopped when the volume of extract was reduced to 1 mL, and then the solvent was further removed under a purified nitrogen stream until the weight was reduced to 50 mg.

**Headspace Isolation by Simultaneous Purging and Extraction (SPE):** The powdered dried leaf of *O. ramonense* (40 g) was placed in a 1 L round bottom flask which was connected to a SPE apparatus developed by Umamo and Shibamoto (7). The flask was kept at 30°C. Headspace volatiles were purged into 200 mL distilled water which was extracted with 30 mL dichloromethane simultaneously for 2 h. After the extract was dried over anhydrous sodium sulfate, the solvent was removed by distillation with a Vigreux column. The distillation was stopped when the volume of extract was reduced to 1 mL, and then the solvent was further removed under a purified nitrogen stream until the weight was reduced to 30 mg.

**Instrumental Analyses of Components:** All samples were analyzed with GC retention indices I (8) and GC/MS. The GC retention index and mass spectral (MS) fragmentation pattern of each component were compared with those of the authentic compound for qualitative analysis. A Hewlett Packard (HP) 5890 Series II gas chromatograph equipped with a 60 m x 0.25 mm ( $d_f = 0.25 \mu\text{m}$ ) DB-Wax bonded-phase fused silica capillary column (J&W Scientific, Folsom, CA) and a flame ionization detector (FID) were used for routine quantitative analysis. The oven temperature was held at 40°C for 2 min and then programmed to 200°C at 2°C/min. Detector and injector temperatures were 250°C. Linear velocity of helium carrier gas flow rate was 30 cm/sec at split ratio of 30/1.

A Varian 3500 GC interfaced to a Finnigan Mat Model 800 ion trap detector was used for MS identification of the GC components. The column and oven conditions were identical to the ones used for a HP GC.

## Results and Discussion

The yield of total volatiles (relative to the dried *O. ramonense* leaves used) were 0.060% (w/w) and 0.093% (w/w) from SDR and SPE, respectively. Table I shows the compounds identified in oils obtained from *Origanum* leaves along with their GC peak area percent and retention indices on a DB-Wax column. Of the total, 132 components were identified.

The compositions of essential oils recovered by two different methods showed significant differences. The oil obtained by SDR contained 42 alcohols which composed 79.5% of the total GC peak area. The major volatile constituents of this oil were  $\alpha$ -terpineol (41.53%), terpinen-4-ol (16.80%), *cis*-sabinene hydrate (13.17%), eugenol (3.61%), and  $\gamma$ -terpinene (3.40%). Volatile compositions of majoram oils vary considerably among different species (1). However, they contain *cis*-sabinene hydrate and/or terpinen-4-ol as the major constituents. For example, the oil obtained from *O. majorana* L. (*syn. Majorana hortensis* Moench) by a simultaneous steam-distillation-extraction method contained *cis*-sabinene hydrate (29.3%) and terpinen-4-ol (20.7%) as the major constituent (9). The oil prepared from *O. majorana* by steam distillation followed by extraction with *n*-pentane also contained *cis*-sabinene hydrate and terpinen-4-ol as the major constituents (10).

It is well known that the method of isolation of volatile compounds has a great influence on the qualitative and quantitative composition of the extracts (11). In particular, the application of elevated temperatures for the distillation causes chemical changes of the original plant components (4). For example, terpinen-4-ol formed from sabinene and sabinene hydrate under elevated temperatures (12). In the present study, steam distillation was conducted at a lower temperature (33°C) than the temperature used in previous reports (9,10). However, some compositional changes must be expected during distillation. Therefore, headspace sampling has been recommended in order to obtain natural plant volatiles (13). One drawback of the headspace sampling method is that amounts of oils recovered are low. Generally, it is almost impossible to separate a sufficient amount of a certain unknown compound from a sample prepared from headspace sampling for further instrumental analysis such as NMR. On the other hand, the SPE method used in the present study provides a sufficient amount of headspace samples for NMR (14). The major constituents of the oil obtained by SPE were 1,8-cineole (17.46%),  $\gamma$ -terpinene (14.69%), terpinen-4-ol (13.88%),  $\alpha$ -terpinene (9.37%), and  $\alpha$ -terpineol (6.83%). The amount of oil obtained by this method was sufficient to perform NMR analysis. For example, the calculated amounts of 1,8-cineole and  $\gamma$ -terpinene obtained were 6.5 mg and 5.5 mg, respectively.

Generally, the oil of *O. ramonense* leaves is a terpene-rich oil. Of the 94 compounds identified in a sample of SDR, 55 were mono- and sesquiterpenes which comprised 91.6% of the total GC peak area. Among 90 compounds identified in a sample of SPE, 57 were mono- and sesquiterpenes which comprised 98.2% of the total GC peak area. Among the 77 terpenoids identified in the present study, caryophyllene derivatives were novel sesquiterpenes in *Origanum*. Figures 1-4 show the structures of four caryophyllene derivatives (along with their mass spectra) found in *O. ramonense* leaf oil in the present study. Caryophylla-2(12),6-dien-5-one and caryophylla-2(12),6(13)-dien-5-one have also been found in lavender oil; it was proposed that they formed from (-)- $\beta$ -caryophyllene upon photo-oxygenation (5). Caryophylla-2(12),6-dien-5-ol and caryophylla-2(12),6(13)-dien-5-ol have been reported in hop oil (15), pepper oil (16), and clove oil (17). In addition, these caryophyllenols were reported in Redwood (*Metasequoia glyptostroboides*) (18) and in allspice (19). The parent compound of the above compounds, caryophyllene was found only in the oil from SPE whereas its oxide derivative was detected in oil from SDR. Also, the above four caryophyllene oxygenated compounds were identified only in the oil from SDR. Therefore, oxygenated caryophyllene derivatives may be formed during steam distillation or they were not recovered by SPE because of their low vapor pressure. On the other hand,  $\beta$ -caryophyllene (14.5%) has recently been reported as one of the major constituents of water-distilled oil of *O. laevigatum* Boiss (20).

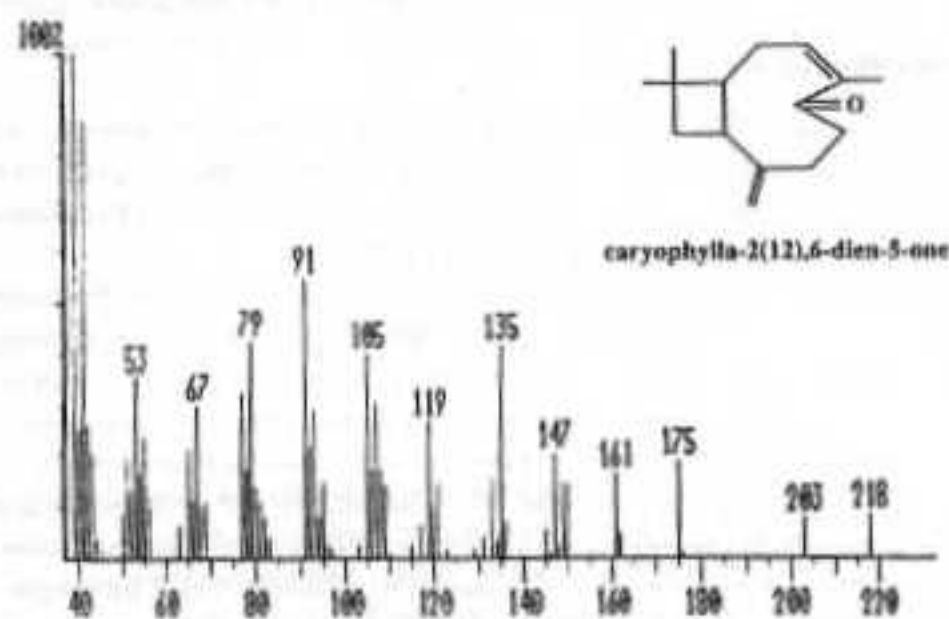


Figure 1. Structure and mass spectra of caryophylla-2(12),6-dien-5-one

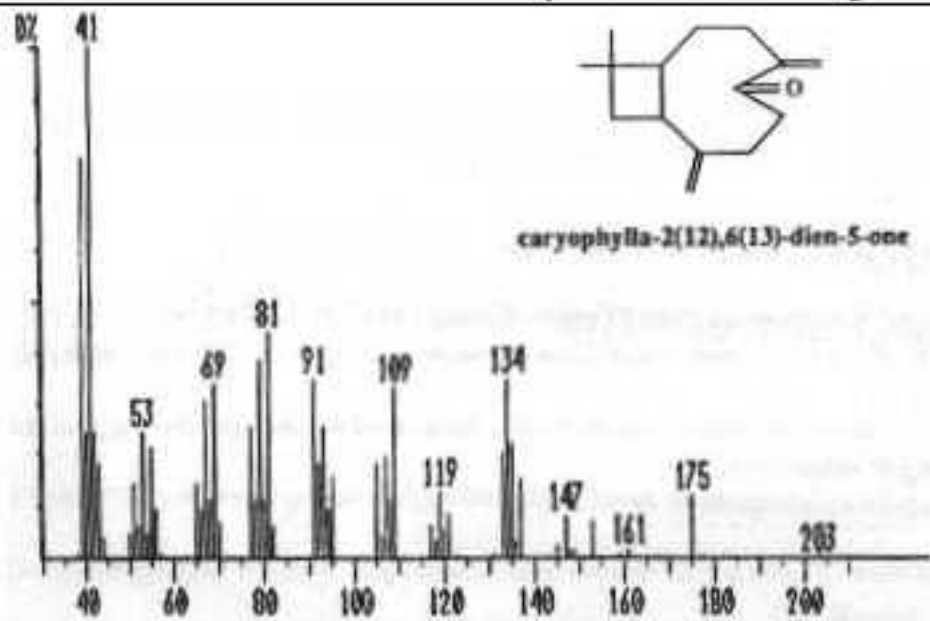


Figure 2. Structure and mass spectra of caryophylla-2(12),6(13)-dien-5-one

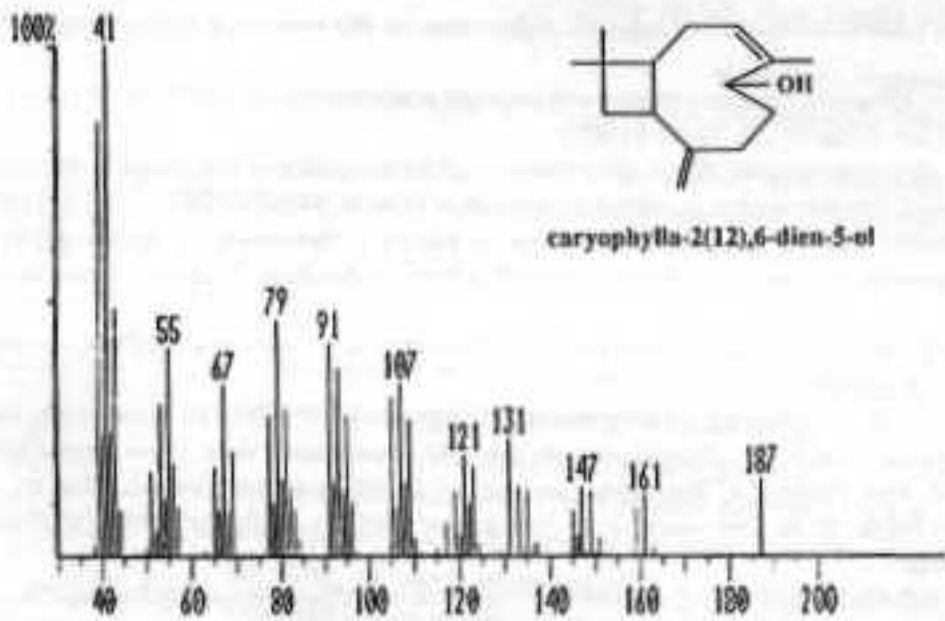


Figure 3. Structure and mass spectra of caryophylla-2(12),6-diene-5-ol

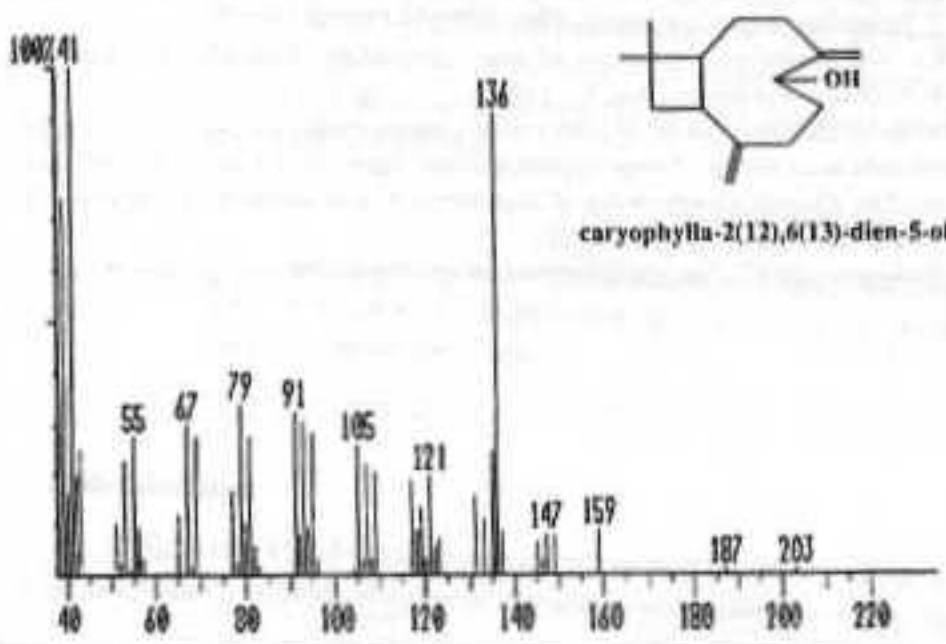


Figure 4. Structure and mass spectra of caryophylla-2(12),6(13)-diene-5-ol

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