Application Note
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Examination of Mass Spectra of Aroma Components in Essential Oils via GC/MS

Using HydroInert Ion Source Specifically for Use with Hydrogen as a Carrier Gas

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Abstract

Essential oils contain many terpene compounds. When a conventional ion source is used with hydrogen as a carrier gas, changes in the mass spectra may be observed for certain compounds. Therefore, the current study examined the characteristics and mechanisms of formation of compounds with mass spectra that are likely to change. Changes suggestive of the dehydration of tertiary alcohols were often observed with compounds. Other changes in mass spectra were observed with compounds containing oxygen such as aldehydes, epoxides, and esters. With the use of the HydroInert ion source, which is specifically for use with hydrogen as a carrier gas, the mass spectra fidelity is retained and allow users to continue to use the existing helium-based mass spectral libraries.

Introduction

GC/MS is often used to analyze aroma components, and helium is commonly used as a carrier gas. However, demand for using hydrogen as a carrier gas is increasing as a result of the recent shortage of helium as well as part of efforts to decarbonize. Aroma components are often analyzed using untargeted analysis (comprehensive analysis), so matching of the retention index (RI) and mass spectrum is an important element of identification.

This Application Note reports the results of a study of the characteristics of compounds with mass spectra that tend to change when using hydrogen carrier gas with a conventional ion source.

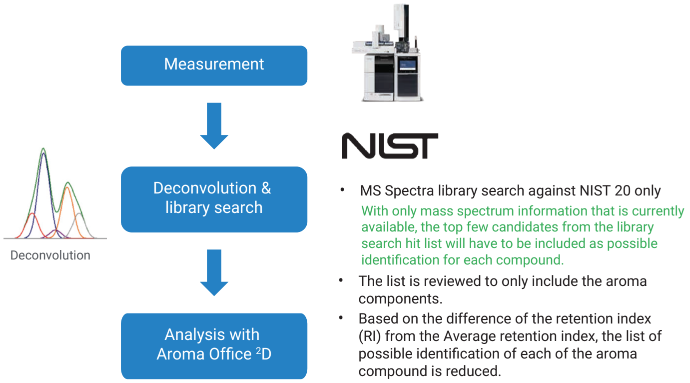
Analytical conditions

13 commercially available essential oils (e.g., rose, hinoki, neroli, peppermint, lemongrass, lemon, ylang-ylang, palmarosa, geranium, and clary sage) were analyzed using hydrogen carrier gas on both conventional ion source and HydroInert ion source.

Instrumentation	: Intuvo 9000GC-5977B GC/MS
Column	: DB-HeavyWAX (p/n 122-7162-INT) Length: 30 m, internal diameter: 0.25 mm, film thickness: 0.25 µm
Injection mode	: Split (200:1)
Inlet temperature	: 250 °C
Guard chip temperature	: Oven track
Bus temperature	: 250 °C
Inlet liner	: Ultra Inert, single taper, low pressure drop, single taper, with wool (p/n 5190-2295)
Injection volume	: 0.2 µL
Column flow	: 1.0 mL/min
Oven temperature	: 80 °C (0 min) - 10 °C/min - 280 °C (10 min)
Transfer line temperature	: 300 °C
Ion source temperature	: 300 °C

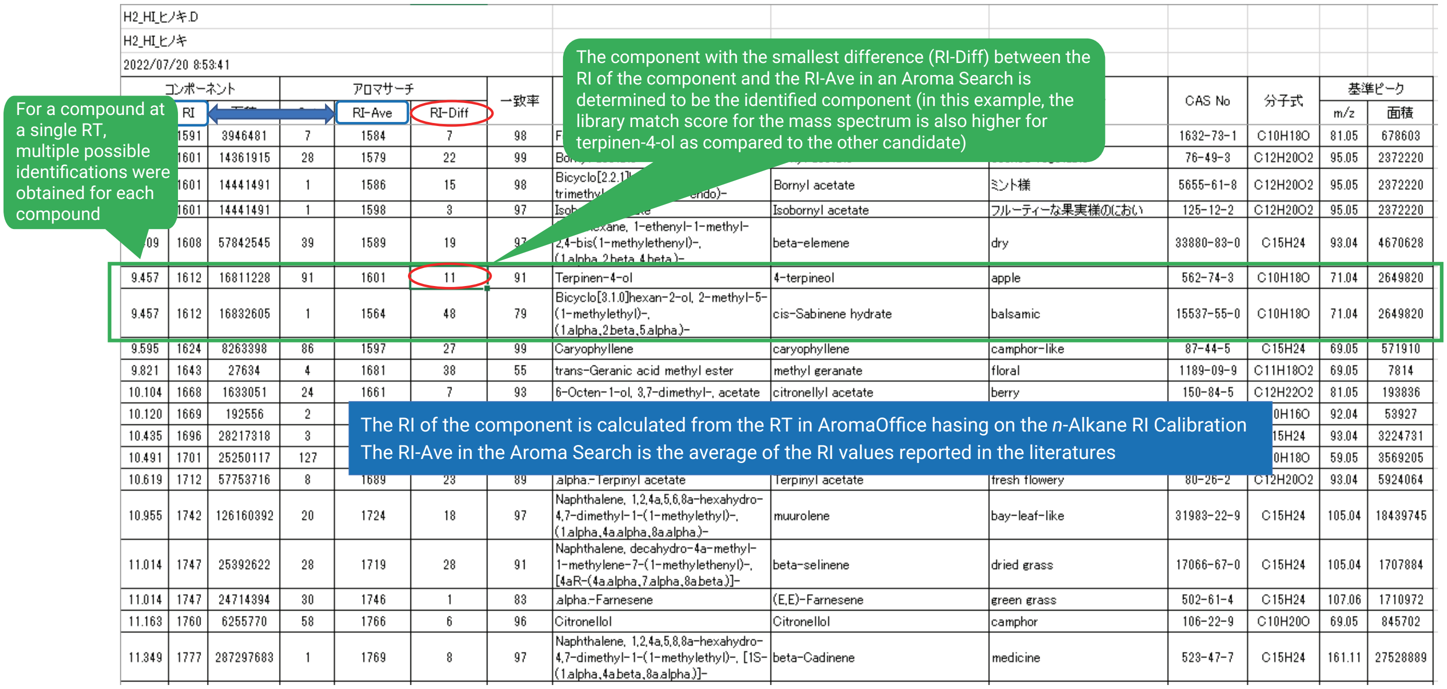
Analytical methods

The data for the 13 Essential Oils were analyzed using Agilent MassHunter Unknowns Analysis, which allows peak detection by deconvolution and library searches. The library used was NIST 20. The results in Agilent Application Note 5994-5751JAJP1) confirmed that RI could be used when using hydrogen carrier gas, so the RI for n-alkane was calculated for the top hit compounds, and then AromaOffice2D was used to narrow down the compounds. The workflow of the analysis of aroma components using AromaOffice2D is shown in Figure 1, and the methods by which compounds were identified are shown in Figure 2.



- MS Spectra library search against NIST 20 only
  With only mass spectrum information that is currently available, the top few candidates from the library search hit list will have to be included as possible identification for each compound.
- The list is reviewed to only include the aroma components.
- Based on the difference of the retention index (RI) from the Average retention index, the list of possible identification of each of the aroma compound is reduced.

Figure 1. Workflow of analysis of aroma components with AromaOffice2D



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コンポーネント			アロマサーチ			一致率				CAS No	分子式	基準ピーク	
	RI			RI-Ave	RI-Diff							m/z	面積
	1591	3946481	7	1584	7	98	F			1632-73-1	C10H18O	81.05	678603
	1601	14361915	28	1579	22	99	Bo			76-49-3	C12H20O2	95.05	2372220
	1601	14441491	1	1586	15	98	Bicyclo[2.2.1] trimethyl endo)-	Bornyl acetate	ミント様	5655-61-8	C12H20O2	95.05	2372220
	1601	14441491	1	1598	3	97	Isob	Isobornyl acetate	フルーティーな果実様のにおい	125-12-2	C12H20O2	95.05	2372220
09	1608	57842545	39	1589	19	97	exane, 1-ethenyl-1-methyl- 2,4-bis(1-methylethenyl)-, (1.alpha.2.beta.4.beta.)-	beta-elemene	dry	33880-83-0	C15H24	93.04	4670628
9.457	1612	16811228	91	1601	11	91	Terpinen-4-ol	4-terpineol	apple	562-74-3	C10H18O	71.04	2649820
9.457	1612	16832605	1	1564	48	79	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-	cis-Sabinene hydrate	balsamic	15537-55-0	C10H18O	71.04	2649820
9.595	1624	8263398	86	1597	27	99	Caryophyllene	caryophyllene	camphor-like	87-44-5	C15H24	69.05	571910
9.821	1643	27634	4	1681	38	55	trans-Geranic acid methyl ester	methyl geranate	floral	1189-09-9	C11H18O2	69.05	7814
10.104	1668	1633051	24	1661	7	93	6-Octen-1-ol, 3,7-dimethyl-, acetate	citronellyl acetate	berry	150-84-5	C12H22O2	81.05	193836
10.120	1669	192556	2								0H16O	92.04	53927
10.435	1696	28217318	3								15H24	93.04	3224731
10.491	1701	25250117	127								0H18O	59.05	3569205
10.619	1712	57753716	8	1689	23	89	.alpha.-Terpinyl acetate	Terpinyl acetate	fresh flowery	80-26-2	C12H20O2	93.04	5924064
10.955	1742	126160392	20	1724	18	97	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)-	muurolene	bay-leaf-like	31983-22-9	C15H24	105.04	18439745
11.014	1747	25392622	28	1719	28	91	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]-	beta-selinene	dried grass	17066-67-0	C15H24	105.04	1707884
11.014	1747	24714394	30	1746	1	83	.alpha.-Farnesene	(E,E)-Farnesene	green grass	502-61-4	C15H24	107.06	1710972
11.163	1760	6255770	58	1766	6	96	Citronellol	Citronellol	camphor	106-22-9	C10H20O	69.05	845702
11.349	1777	287297683	1	1769	8	97	Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1S-(1.alpha.,4a.beta.,8a.alpha.)]-	beta-Cadinene	medicine	523-47-7	C15H24	161.11	27528889

For a compound at a single RT, multiple possible identifications were obtained for each compound

The component with the smallest difference (RI-Diff) between the RI of the component and the RI-Ave in an Aroma Search is determined to be the identified component (in this example, the library match score for the mass spectrum is also higher for terpinen-4-ol as compared to the other candidate)

The RI of the component is calculated from the RT in AromaOffice hasing on the *n*-Alkane RI Calibration
The RI-Ave in the Aroma Search is the average of the RI values reported in the literatures

Figure 2. Methods by which compounds were identified with AromaOffice2D

Results

With the use of hydrogen carrier gas on a conventional ion source, many compounds were observed to have low match score against the library. The aroma components identified by AromaOffice2D with a NIST search match score of at least 90 % with the use of HydroInert source were being selected for comparison of the corresponding NIST search match score with the conventional ion source when using hydrogen carrier gas. As shown in Figure 3, 20-40 % of the list of aroma components showed a corresponding match score of lower than 60 % when the conventional ion source was used.

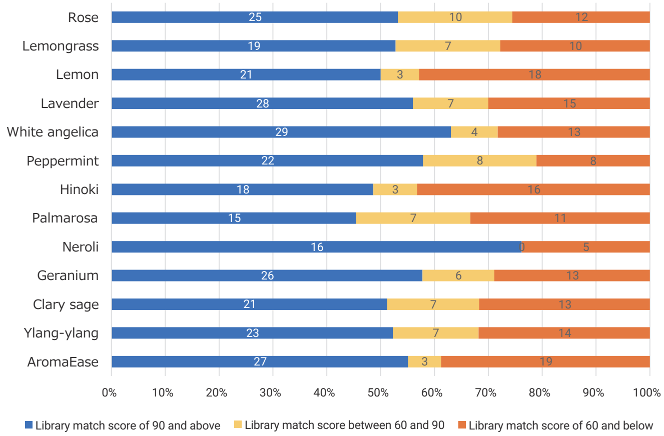


Figure 3. Distribution of the match score for aroma components with mass spectra obtained with a conventional ion source (H_2). These aroma components with mass spectra matched at a match score of 90 % or higher with the HydroInert ion source (H_2)

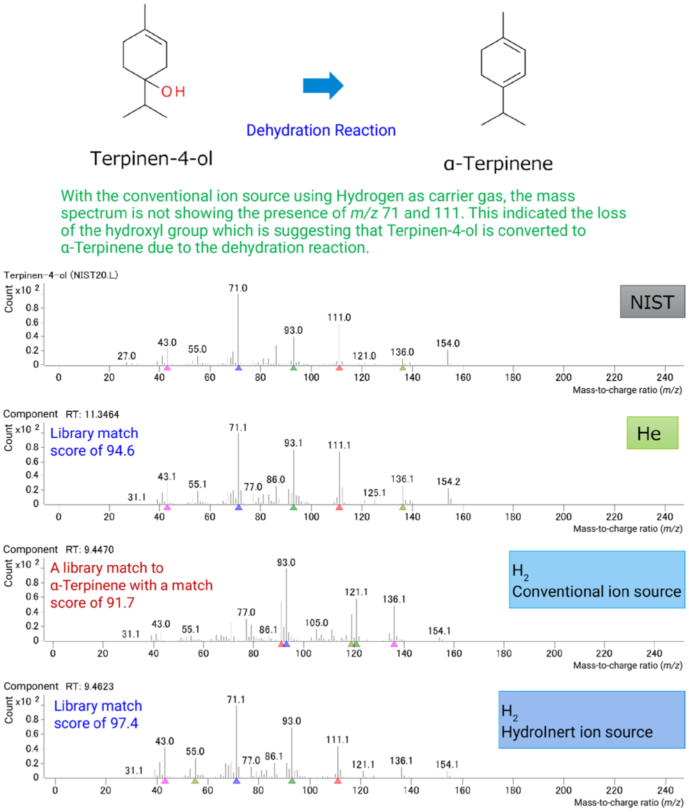
Compounds with mass spectra changed markedly when a conventional ion source is used include monoterpene alcohols such as linalool, geraniol, and terpinen-4-ol, sesquiterpene alcohols such as bisabolol, aldehydes such as neral, and epoxide structures such as limonene oxide. Among terpene alcohols, tertiary alcohols in particular had a substantial change in their mass spectra and the disappearance of ions with hydroxyl groups from their mass spectra suggested that the mass spectra changed due to dehydration reaction.

Terpinen-4-ol in peppermint is an example of a terpene alcohol with a substantial change in its mass spectrum. Its structural formula and a comparison of its mass spectrum with each ion source/carrier gas are shown in Figure 4. When hydrogen was used as a carrier gas with a conventional ion source, the mass spectra was library matched with a high score to α-terpinene which is the dehydration reaction product of terpine-4-ol.

In addition, the structural formula and comparison of mass spectrum with each ion source/carrier gas for linalool in lemon grass and neral and limonene oxide in lemon are shown in Figures 5-7, respectively.

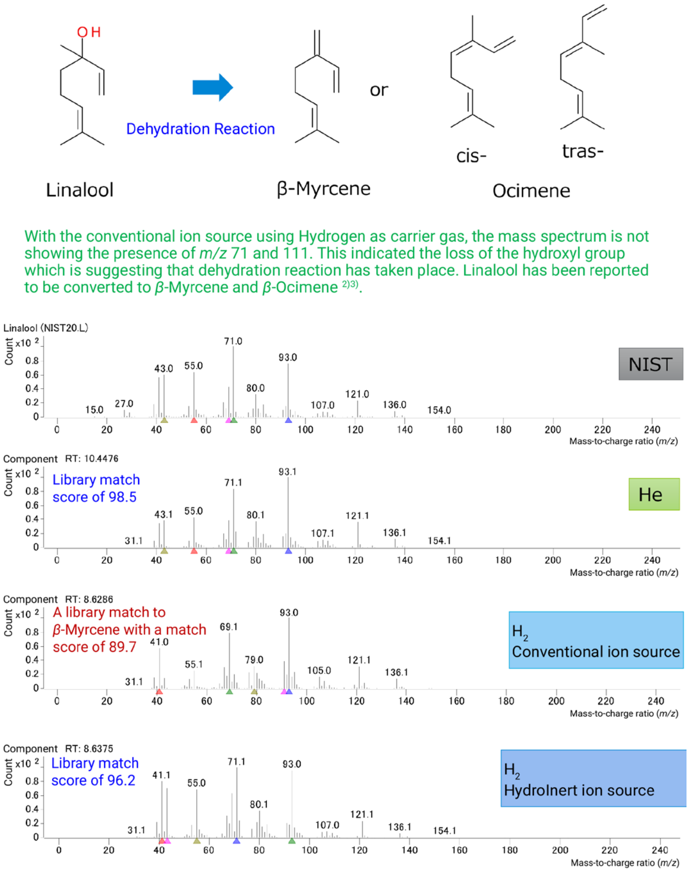
That said, almost no changes in mass spectra in comparison with the helium-based NIST library were observed when using hydrogen carrier gas with the HydroInert ion source, suggesting that these reactions occurred inside the ion source and were suppressed by the HydroInert ion source.

Compounds with mass spectra that changed with use of hydrogen carrier gas with the conventional ion source are summarized by group in Figure 8.



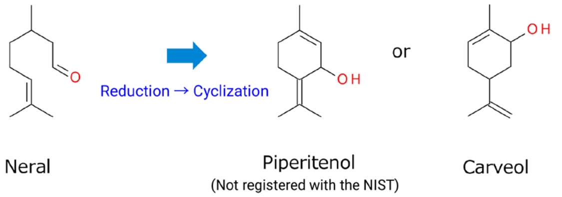
With the conventional ion source using Hydrogen as carrier gas, the mass spectrum is not showing the presence of *m/z* 71 and 111. This indicated the loss of the hydroxyl group which is suggesting that Terpinen-4-ol is converted to α-Terpinene due to the dehydration reaction.

Figure 4. Terpinen-4-ol in peppermint



With the conventional ion source using Hydrogen as carrier gas, the mass spectrum is not showing the presence of *m/z* 71 and 111. This indicated the loss of the hydroxyl group which is suggesting that dehydration reaction has taken place. Linalool has been reported to be converted to *β*-Myrcene and *β*-Ocimene [2)3)].

Figure 5. Linalool in lemongrass



With the conventional ion source using Hydrogen as the carrier gas, a library match of the compound to Carveol suggesting that the Neral (aldehyde) was reduced to an alcohol and then cyclized.

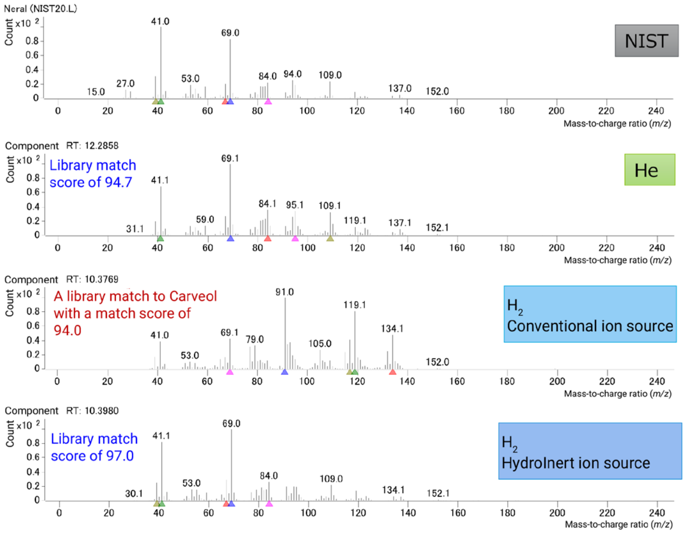
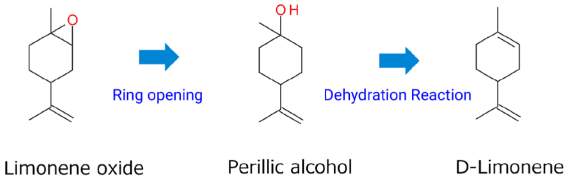


Figure 6. Neral in lemon



A library match of Perillic Acid when using the conventional ion source with Hydrogen as carrier gas suggested the opening of the Epoxide ring. After ring opening, dehydration reaction may also occur and it is further converted to limonene.

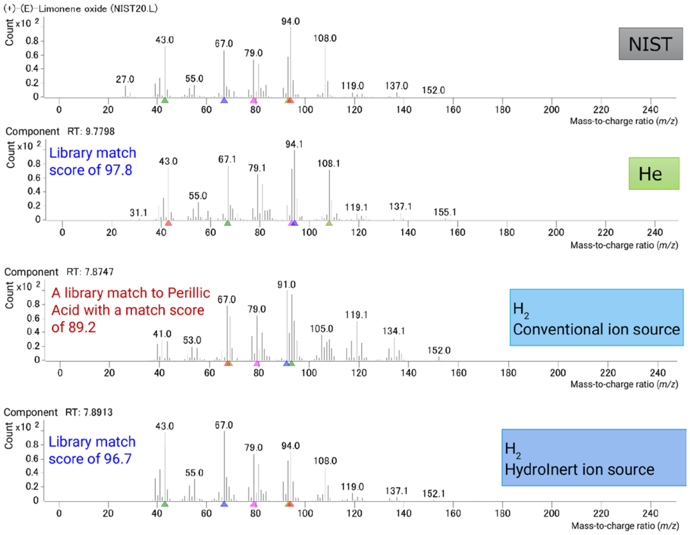


Figure 7. Limonene oxide in lemon

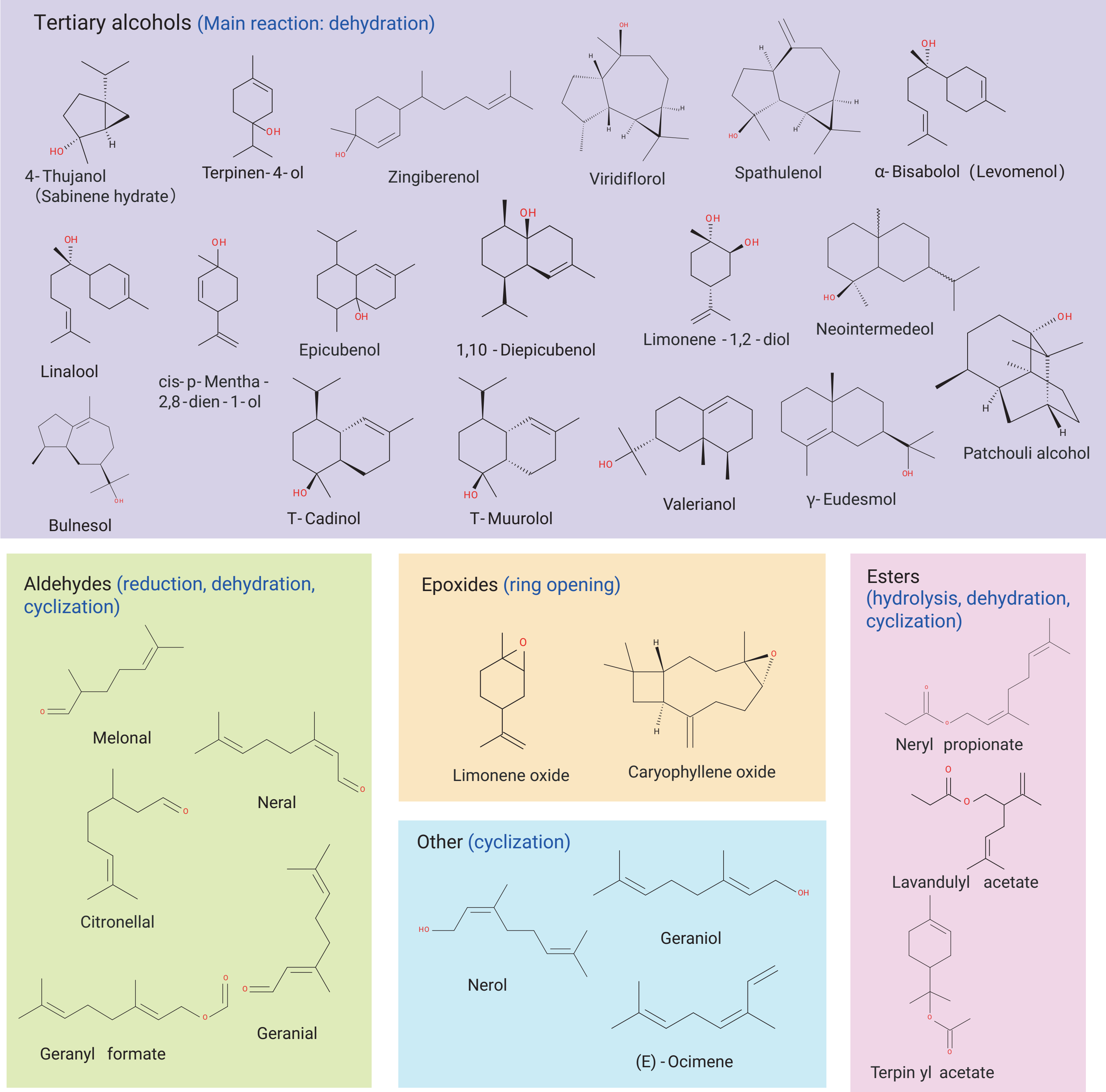


Figure 8. Compounds with substantial changes in their mass spectra when using a conventional ion source (H_2)

Conclusion

Changes in mass spectra for aroma compounds when the carrier gas was changed from helium to hydrogen in GC/MS were examined. The results showed that the mass spectra of some compounds were observed to change when using hydrogen carrier gas on the conventional ion source. Terpene alcohols are abundantly found in essential oils. Changes in mass spectra tended to be substantial for those alcohols and for tertiary alcohols in particular. Changes in mass spectra were also observed with compounds containing oxygen such as aldehydes, epoxides, and esters. Although not shown here, the mass spectra of some compounds changed to a slightly different extent depending on the amount and concentration of other components in the essential oil.

Use of the HydroInert ion source produced mass spectra for these compounds that were comparable to those produced with the conventional ion source using helium carrier gas, indicating the effectiveness of the HydroInert ion source in GC/MS qualitative analysis with hydrogen carrier gas.

References

1. Evaluation of Odor Database via GC/MS Using HydroInert Ion Source Specifically for Use with Hydrogen as a Carrier Gas, *Agilent Technologies application note*, publication number 5994-5751JAJP
2. E. Jakab *et al.*, Thermo-oxidative decomposition of lime, bergamot and cardamom essential oils, J. Analytical and Applied Pyrolysis 134 (2018) 552-561
3. Hui-Ting Chang *et al.*, Thermal Degradation of Linalool-Chemotype Cinnamomum osmophloeum Leaf Essential Oil and Its Stabilization by Microencapsulation with β-Cyclodextrin, Molecules 2021, 26, 409

This Application Note includes content presented at the 66th Symposium on the Chemistry of Terpenes, Essential oils and Aromatics in 2022.

Note: Handling of hydrogen gas

Hydrogen gas is a gas that can ignite and explode when mixed with oxygen. Users are responsible for handling hydrogen gas with the utmost care in the operating environment, including the hydrogen gas source, the analytical instrument into which the gas is introduced and the intermediate piping, and during the operation of the instrument.

For more detailed information on each GC system, please refer to the following technical overviews.

Technical Overview: Hydrogen Safety for the Agilent 8860 GC System
(Publication number 5994-5419EN)
Hydrogen Safety for the Agilent 8860 GC System

Technical Overview: Hydrogen Safety for the Agilent 8890 GC System
(Publication number 5994-5413EN)
Hydrogen Safety for the Agilent 8890 GC System

Technical Overview: Hydrogen Safety for the Agilent Intuvo 9000 GC System
(Publication number 5994-5412EN)
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