Available online at www.sciencedirect.com
ScienceDirect
Mendeleev Commun., 2013, **23**, 51–52

Mendeleev
Communications

Characterization of the chemical constituents of agarwood oils from Malaysia by comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry

Saiful Nizam Tajuddin,[a] Norfatirah S. Muhamad,[a] Mohd A. Yarmo[b] and Mashitah M. Yusoff *[a]

[a] *Faculty of Industrial Sciences and Technology, University Malaysia Pahang, 26300 Gambang, Pahang, Malaysia. Fax: +609 549 2766; e-mail: saifulnizam@ump.edu.my, annexefistlab@gmail.com*
[b] *Faculty of Science and Technology, University Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia*

DOI: 10.1016/j.mencom.2013.01.019

4-Phenyl-2-butanone, α-bulnesene, α-guaiene, agarospirol, ledene oxide-(II), elemol and γ-eudesmol were identified as the major chemical constituents of Malaysian agarwood oils.

Comprehensive two-dimensional gas chromatography (GC×GC) arose from the need for a more powerful and reliable solution to separation and resolution problems encountered in very complex samples.[1–5] This technique has been applied to the analysis of food, flavour, fragrance and environmental samples.[6–10] A time-of-flight mass spectrometric (TOF-MS) detector is preferred for GC×GC due to a higher collection frequency.[11] Mass spectrometry is often insufficient to distinguish between structural isomers as the defragmentation patterns are often identical.[12] The combined GC×GC/TOF-MS technique is able to deconvolute chromatographic peaks for better identification and quantification.[13]

Agarwood is a scented product obtained from a pathological condition of the wood of standing trees of *Aquilaria* species (*Thymelaeaceae*). *Aquilaria malaccensis* is the major source of agarwood used as perfume and incense ingredients in Malaysia.[14,15] The purpose of this study was to analyze the volatile compounds of Malaysian agarwood oil using GC×GC/TOF-MS and to identify the components of complex mixtures of terpene compounds in the oil.

Figure 1 shows the GC×GC contour plots of oil under different column systems and optimized separation conditions.[†] Peaks were identified using the NIST library (version 2.0), and Table 1

Table 1 Chemical compounds identified in agarwood oil using GC×GC/TOF-MS.

Peak	Component	Retention time/s	TIC	Molecular formula	Similarity	Area (%)	CAS no.
1	Benzaldehyde	430	1.030	C_7H_6O	965	1.93	100-52-7
2	2-Hydroxybenzaldehyde	570	1.205	$C_7H_6O_2$	949	0.35	90-02-8
3	Acetophenone	615	1.345	C_8H_8O	972	0.22	98-86-2
4	4-Phenyl-2-butanone	1015	1.360	$C_{10}H_{12}O$	930	5.83	2550-26-7
5	Copaene	1305	0.760	$C_{15}H_{24}$	888	0.13	3856-25-5
6	α-Cedrene	1390	0.760	$C_{15}H_{24}$	850	0.12	469-61-4
7	*trans*-α-Bergamotene	1400	0.780	$C_{15}H_{24}$	883	0.12	0-00-0
8	Caryophyllene	1410	0.770	$C_{15}H_{24}$	844	0.12	87-44-5
9	α-Guaiene	1455	0.790	$C_{15}H_{24}$	912	0.19	3691-12-1
10	α-Caryophyllene	1535	0.780	$C_{15}H_{24}$	822	0.19	6753-98-6
11	α-Selinene	1565	0.795	$C_{15}H_{24}$	871	0.16	473-13-2
12	β-Selinene	1580	0.775	$C_{15}H_{24}$	893	0.14	17066-67-0
13	δ-Cadinene	1650	0.785	$C_{15}H_{24}$	863	0.14	483-76-1
14	Alloaromadendrene	1745	1.040	$C_{15}H_{24}$	887	0.12	25246-27-9
15	Aromadendrene	1835	0.990	$C_{15}H_{24}$	834	0.12	109119-91-7
16	α-Muurolene	1840	1.025	$C_{15}H_{24}$	844	0.97	31983-22-9
17	α-Bulnesene	1870	1.150	$C_{15}H_{24}$	829	2.47	3691-11-0
18	(–)-Aristolene	1880	1.035	$C_{15}H_{24}$	821	0.49	6831-16-9
19	γ-Gurjunene	1890	1.130	$C_{15}H_{24}$	846	1.96	22567-17-5
20	Elemol	1905	1.070	$C_{15}H_{26}O$	816	2.35	639-99-6
21	1,2-Epoxide-humulene	1920	0.925	$C_{15}H_{24}O$	810	0.42	19888-34-7
22	δ-Gurjunenepoxide	1925	0.790	$C_{15}H_{24}O$	858	0.26	184705-51-9
23	γ-Eudesmol	1945	1.045	$C_{15}H_{26}O$	836	2.68	1209-71-8
25	Agarospirol	1945	1.135	$C_{15}H_{26}O$	851	2.08	1460-73-7
26	Isoaromadendrene epoxide	2050	1.190	$C_{15}H_{24}O$	816	0.68	0-00-0
27	*cis*-Nerolidol	2080	1.075	$C_{15}H_{26}O$	819	0.19	142-50-7
28	Ledene oxide-(II)	2100	1.295	$C_{15}H_{24}O$	822	2.17	0-00-0
29	*trans*-Longipinocarveol	2105	1.255	$C_{15}H_{24}O$	810	2.01	0-00-0
30	Longipinocarvone	2270	1.130	$C_{15}H_{22}O$	830	0.23	0-00-0

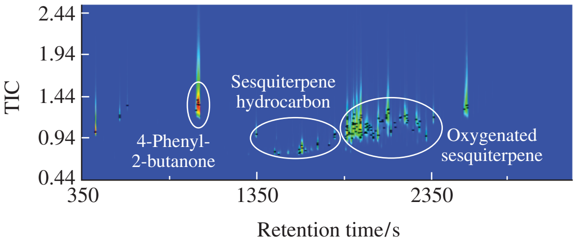

Figure 1 The GC×GC/TOF-MS contour plot of agarwood oil on column system in Table 1, with 30 marker components.

summarizes the results. The mass spectral match factors include similarity, reverse, and probability. The similarity and reverse factors indicate how well a mass spectrum matches the library spectrum, but isomers have similar mass spectra, so probability was used to determine whether the peaks with the same name belong to one compound or several compounds. According to published data[16] a similarity and reverse number above 800 and 900, respectively, indicate that an acquired mass spectrum usually is a good match with the library spectrum.

It can be seen that an apparent group-type separation of some major components in the sample was obtained using this method. The components identified as terpene, oxygenated terpene, sesquiterpene and oxygenated sesquiterpene were located at different retention times. Owing to the lack of pure compounds, it is difficult to confirm the GC×GC/TOF-MS identification for all of the individual compounds. In total, more than 300 compounds were identified, but only 30 compounds with similarity and reverse numbers above 800 and 900 are listed (Table 1). However, several major constituents with special aroma and high intensity, such as alloaromadendrene, valencene, γ-gurjunene, β-guaiene, eremophilene, α-selinene, agarospirol and γ-eudesmol, were clustered in a complex mixture.

In conclusion, GC×GC/TOF-MS is an effective tool in separating the constituents of a very complex mixture such as the Malaysian agarwood oil. When coupled to MS-TOF, the GC×GC system, column sets, sample and modulation system showed an improvement in sensitivity and resolution. As a result, the similarity and reverse values were higher for MS-TOF than for MS, yielding superior mass spectra such as that only found in reference libraries. Another consequence of this higher sensitivity and resolution was the gain in terms of number of peaks tentatively identified in the Malaysian agarwood oil when a GC×GC/TOF-MS technique was employed.

This work was supported by the Malaysian Ministry of Science, Technology and Innovation (Science Fund MOSTI 02-01-16-SF0005) awarded to Mashitah M. Yusoff.

References

1 L. Mondello, P. Q. Tranchida, P. Duga and G. Dugo, *Mass Spectrom. Rev.*, 2008, **27**, 101.
2 P. Marriot, R. Shellie and C. Cornwell, *J. Chromatogr. A*, 2001, **936**, 1.
3 J. Wu, X. Lu, W. Tang, H. Kong, S. Zhou and G. Xu, *J. Chromatogr. A*, 2004, **1034**, 199.
4 J. Beens and U. A. Th. Brinkman, *Analyst*, 2005, **130**, 123.
5 M. Adahchour, J. Beens and U. A. Th. Brinkman, *J. Chromatogr. A*, 2004, **1186**, 67.
6 E. M. Rachel, M. D. Kenneth, C. H. Jamin and T. Y. Elton, *Anal. Chem.*, 2006, **78**, 2700.
7 J. M. Sanchez and R. D. Sacks, *Anal. Chem.*, 2006, **78**, 3046.
8 M. P. Karisa, L. H. Janiece, C. H. Jamin and R. E. Synovec, *Talanta*, 2006, **70**, 797.
9 P. Ognjen and G. Tadeusz, *Anal. Bioanal. Chem.*, 2006, **386**, 1013.
10 K. Bester, *J. Chromatogr. A*, 2009, **1216**, 470.
11 K. Wang, R. Zhu, R. Qu and Z. Li, *Mendeleev Commun.*, 2012, **22**, 45.
12 (*a*) M. Šanda and P. Žáček, http://www.sciencedirect.com/science/article/pii/S1570023211007847-fn0005; (*b*) L. Streinz, M. Dračínský and B. Koutek, *J. Chromatogr. B*, 2012, **881**, 69.
13 P. Marriott and R. Shellie, *Trends Anal. Chem.*, 2002, **21**, 573.
14 A. Barden, N. Awang Anak, T. Mulliken and M. Song, *Heart of the Matter. Agarwood Use and Trade and CITES Implementation for Aquilaria malaccensis*, TRAFFIC International, Cambridge, 2000.
15 L. T. Ng, Y. S. Chang and A. A. Kadir, *J. Trop. Forest Prod.*, 1997, **2**, 272.

Received: 18th September 2012; Com. 12/3980

[†] The GC×GC/TOF-MS system consisted of an Agilent 6890 gas chromatograph, a Pegasus IV time-of-flight mass spectrometer (Leco Corp., St Josephs, MI) and a cold-jet modular KT-2001 Retrofit prototype (Zoex Corp., Lincoln, NE). Agilent DB-1 (30 m × 0.25 mm × 0.1 μm) and Agilent DB-Wax (1.0 m × 0.1 mm × 0.1 μm) columns were used; helium was a carrier gas (a constant pressure of 607 kPa); the modulation time was 5 s. The columns were held at 60 °C for 0.5 min and then heated at a rate of 3 K min^{-1} to 230 °C and held for 10 min. Ethyl acetate was used as a solvent to dilute the analyte.