ANALYSIS OF NON POLAR FRACTION FROM MAHKOTA DEWA (*Phaleria macrocarpa (Scheff.) Boerl.*) FRUIT WITH GAS CHROMATOGRAPHY-MASS SPECTROSCOPY

Susilawati[1)], Sabirin Matsjeh[2)], Harno Dwi Pranowo[2)], Chairil Anwar[2)]

[1)]Student of Doctoral Program, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Gadjah Mada University, Sekip Utara, Yogyakarta, Indonesia,
[2)]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Gadjah Mada University, Sekip Utara, Yogyakarta, Indonesia
Telp : +6281268731997, e-mail : wati.susila@ymail.com

ABSTRACT

Analysis of n-hexane fraction from mahkota dewa fruit had been carried out by using GC-MS. The result of GC separation revealed that n-hexane fraction contains 27 component but only 8 component were identified. Based on the GC-MS library prediction, compounds from peak 2, 3, 6, 7, 8, 20, 22 and 25 respectively were analysed as styrene **(1)**, furan, tetrahydro-2,2-dimethyl **(2)**, phenol, 2-methoxy-4-(2-propenyl)-eugenol **(3)**, 2-propanoic acid 3-phenyl methyl ester (methyl cinnamic) **(4),** benzene 1,2-dimethoxy-propenyl 4-2 metileugenol **(5)**, hexadecanoic acid methyl ester (methyl palmitic) **(6)** methyldihydromalvalic **(7)** tetracosanoic acid, methyl ester **(8).** Unfortunately another peak unidentified. Analysis fragmentation were carried out for the 3 compounds that have been successfully identified.

Key words : Fruit of mahkota dewa, *Phaleria macrocarpa* (Scheff.) Boerl), Thymelaeaceae

INTRODUCTION

Mahkota dewa plant (*Phaleria macrocarpa* (Scheff.) Boerl.*)* a Thymelaeaceae, is much found in Indonesia. Mahkota dewa is classified as plant capable of living in various conditions, from lowland to highland. This plant can be grown in the garden and also in the pot. Its cultivation is easily done in either vegetative or generative processes. Its productivity was able to reach tens of years. This plant has synonym of *Phaleria papuana var warb wichnanmi* (val) Back. Its trade name in English is crown of God. The name of this plant in Sumatra (Malay) and Depok is *simalakama*. In Java, it is also called as *makutadewa*, *makuto rajo*, *makuto ratu* or *makuto mewo* (Harmanto, 2005). Mahkota dewa fruit is most frequently and empirically utilized by Indonesian various diseases treatment with satisfactory results (Sumastuti and Sonlimar, 2006).

Chemical content of semi-polar and polar fraction of mahkota dewa fruit already known from the literature. They were icariside C_3 (glycosides sesquiterpen) **(A),** glucoside benzophenone **(B)** and mangiferin (xanton glycosides) **(C)** of the chloroform fraction ripe fruit of the mahkota dewa (Oshimi, *et al.*, 2008). In addition, it has been isolated as a glucoside benzophenone **(B)** from ethyl acetate extract of fruit of mahkota dewa and have bioactivity with DPPH (α,α-diphenyl-β-picrylhydrazyl) and P-388 murine cells (Hakim *et al.*, (2004). Glycoside benzophenone has been also isolated by Tambunan and Simanjuntak (2006) from n-butanol extract of the fruit of mahkota dewa. Lignan **(D)** has been isolated from the ethyl acetate fraction of mahkota dewa fruit (Lisdawati *et al.*, 2007).

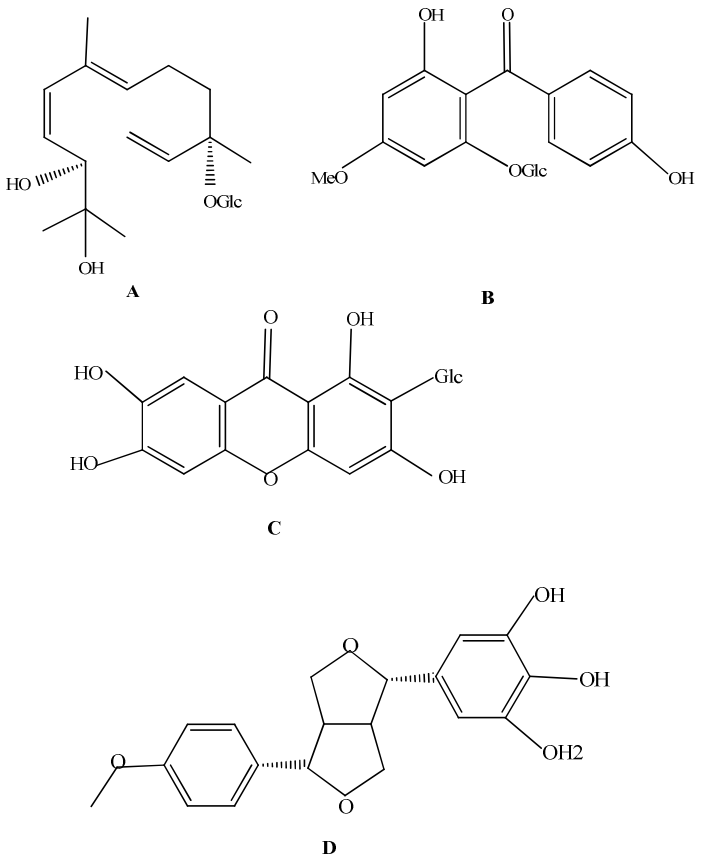


Figure 1 Structures of compounds isolated from semi-polar fraction of mahkota dewa fruit

Non polar content of mahkota dewa have not information yet from the literature. And when the mahkota dewa utilize herbs may be components were carried into the body. Therefore important to identify compounds that become a component of non-polar fraction this mahkota dewa. Non-polar compounds such as malvalat, fatty acid esters, eugenol, furan and styrene easily evaporate into a gas that can be identified by gas chromatography.

The purpose of this study was to identify chemical compounds from non-polar fraction of mahkota dewa fruit with GC-MS instrument. This research is expected to provide information about the content or compound of the n-hexane fraction of mahkota dewa fruit and make a positive contribution in the development of organic chemistry of natural products.

EXPERIMENTS

Plant Material: Fruit of mahkota dewa *(Phaleria macrocarpa* (Scheff.) Boerl) was collected from campus of Gadjah Mada University, Yogyakarta Indonesia in January 2009. The plant was identified by Plant Taxonomy Laboratory, Faculty of Biology, Gadjah Mada University.

Equipment and Materials

A. Equipment used:

Glass tools commonly used in laboratory, analytical balance, a set of tools distillation (simple distillation and vacuum distillation) maserator (drip pans), rotary evaporator (Buchii R-124), separating funnel, Whatman paper No 1, GC-MS-QP2010S Shimadzu

B. The materials required:

Mahkota dewa fruit, methanol (technical), n-hexane (technical), chloroform p.a (Merck) and ethyl acetate (technical).

Research conducted over six months at the Laboratory of Organic Chemistry Department of Chemistry, Gadjah Mada University Yogyakarta

Procedure

A. Sample preparation

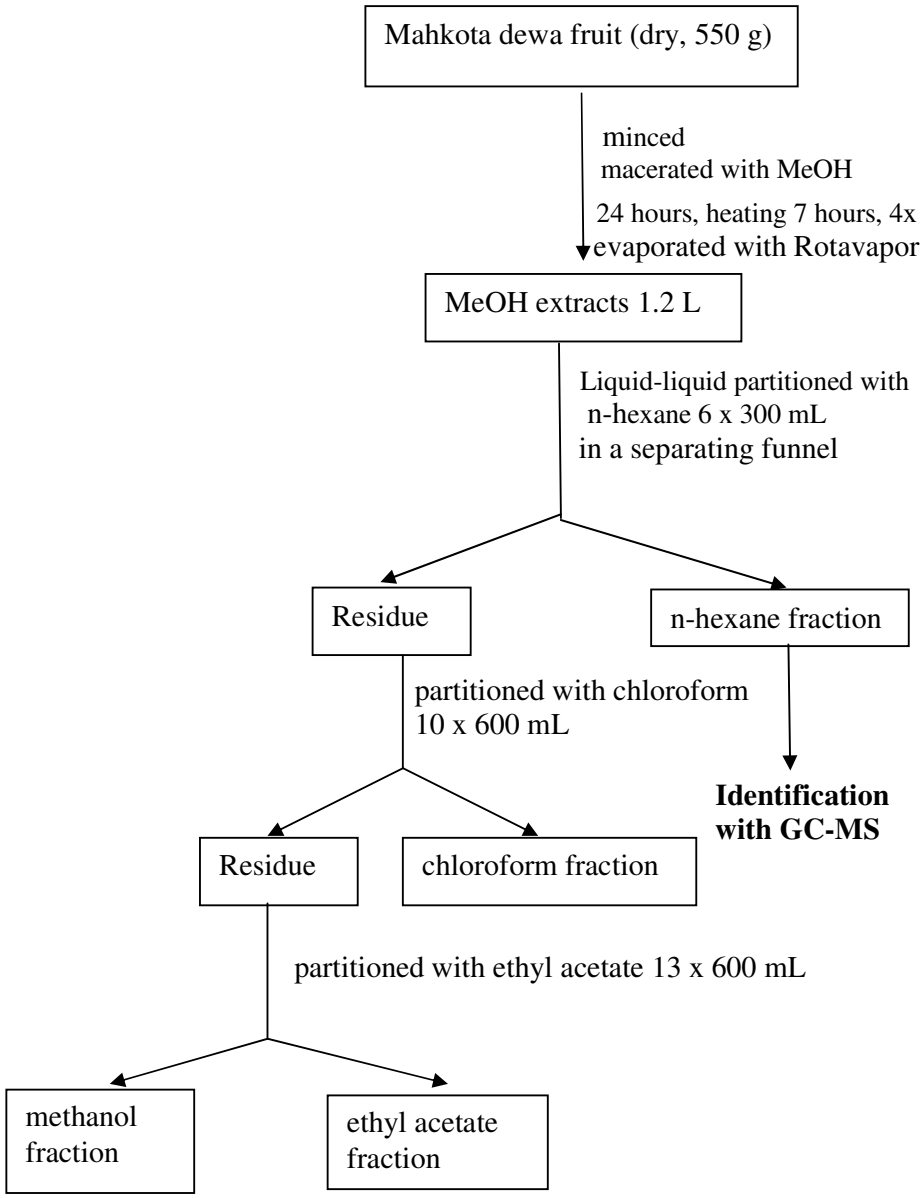
Mahkota dewa fruit (10 kg), cleaned, cut into small pieces and dried for several days in open space and protected from sunlight, after the dried sample is ready for further treatment.

B. Extraction and Fractionation

The samples were dried for several days aerating extracted using macerator with methanol by heating (60^{o}C) for 7 hours then allowed to cool at room temperature for up to 24 h. The residue was macerated for 4 times. The collected methanol extract was concentrated using rotary evaporator to evaporate the solvent in order to obtain concentrated methanol extracts.

Liquid-liquid partition were performed using separating funnel with n-hexane, chloroform and ethyl acetate. Concentrated methanol extract (1.2 L) with less fractionated respectively with n-hexane (6 x 300 mL), chloroform (10 x 600 mL) and ethyl acetate (13 x 600 mL). Then all fractions evaporated by rotary evaporator to obtain dry fraction. Chloroform fraction, ethyl acetate fraction and methanol fraction followed by the chromatography column for further separation and purification (not studied in this seminar). The n-hexane fraction containing components that evaporate easily be identified by GC-MS.

Work Scheme of Extraction



C. GC-MS analysis

Measurement of GC-MS spectrum-Shimadzu QP2010S done on the condition of equipment as follows: injector temperature 320 ° C, column temperature of 250 ° C, RTx-5ms column, column length 30 meters, column diameter of 12:25 mm, temperature rise 70 ° C, carrier gas helium, flow rate 0.50 mL / min, type MSD detector, the speed of paper in 1250.

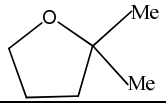
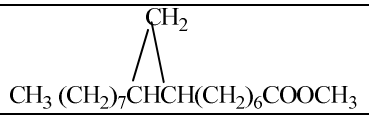
RESULTS AND DISCUSSION

Methanol extract (reddish brown) was obtained from ± 550 g of sample. Concentrated methanol extract (1,2 L) was obtained after evaporation. Concentrated methanol extract was fractionated with non-polar to polar solvents namely n-hexane, chloroform and ethyl acetate to give the n-hexane fraction (light yellow transparent), chloroform fraction (turbidy white on separating funnel, light yellow transparent on the bottle), ethyl acetate fraction (transparent yellow) and methanol fraction. After evaporation with a rotary evaporator equipment obtained 3.25 g of dry n-hexane fraction, 5.55 g of chloroform fraction, 27 g of ethyl acetate fraction and 9.15 g of methanol fraction. n-Hexane fraction was identified by GC-MS, the other fraction continued to chromatography columns for isolated compounds.

Analysis of the n-hexane fraction of mahkota dewa fruit by Gas Chromatography-Mass Spectrometer (GC-MS)

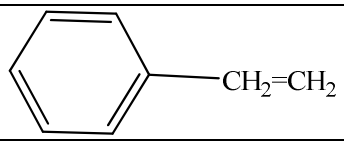
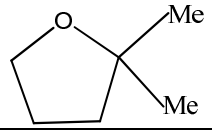
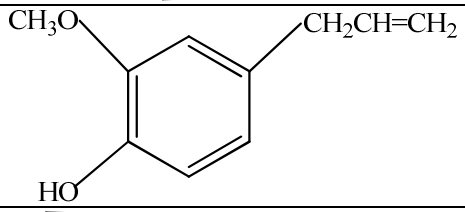
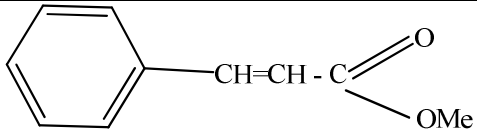
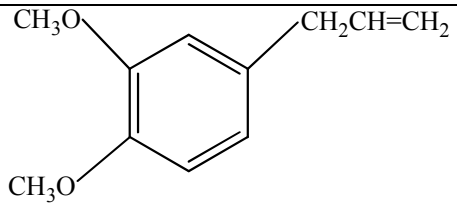
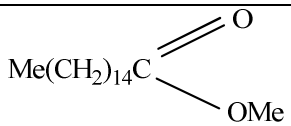
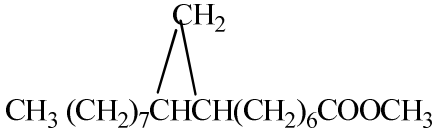
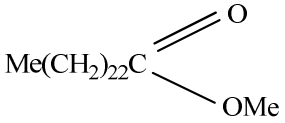
Analysis using gas chromatography-mass spectrometry (GC-MS) aims to determine the structure and molecular weight of a compound from fragmentation resulting. GC chromatogram from n-hexane fraction of mahkota dewa fruit was generated 27 peak (presented in Table 1 and Appendix 1). Of the 27 peak is only eight peak can be identified by MS. Identification of chemical components was performed with compared patterns of the sample mass spectrum with mass spectral patterns of contained in the data base.

Table 1 Results Identification of n-hexane fraction of mahkota dewa fruit with GC-MS

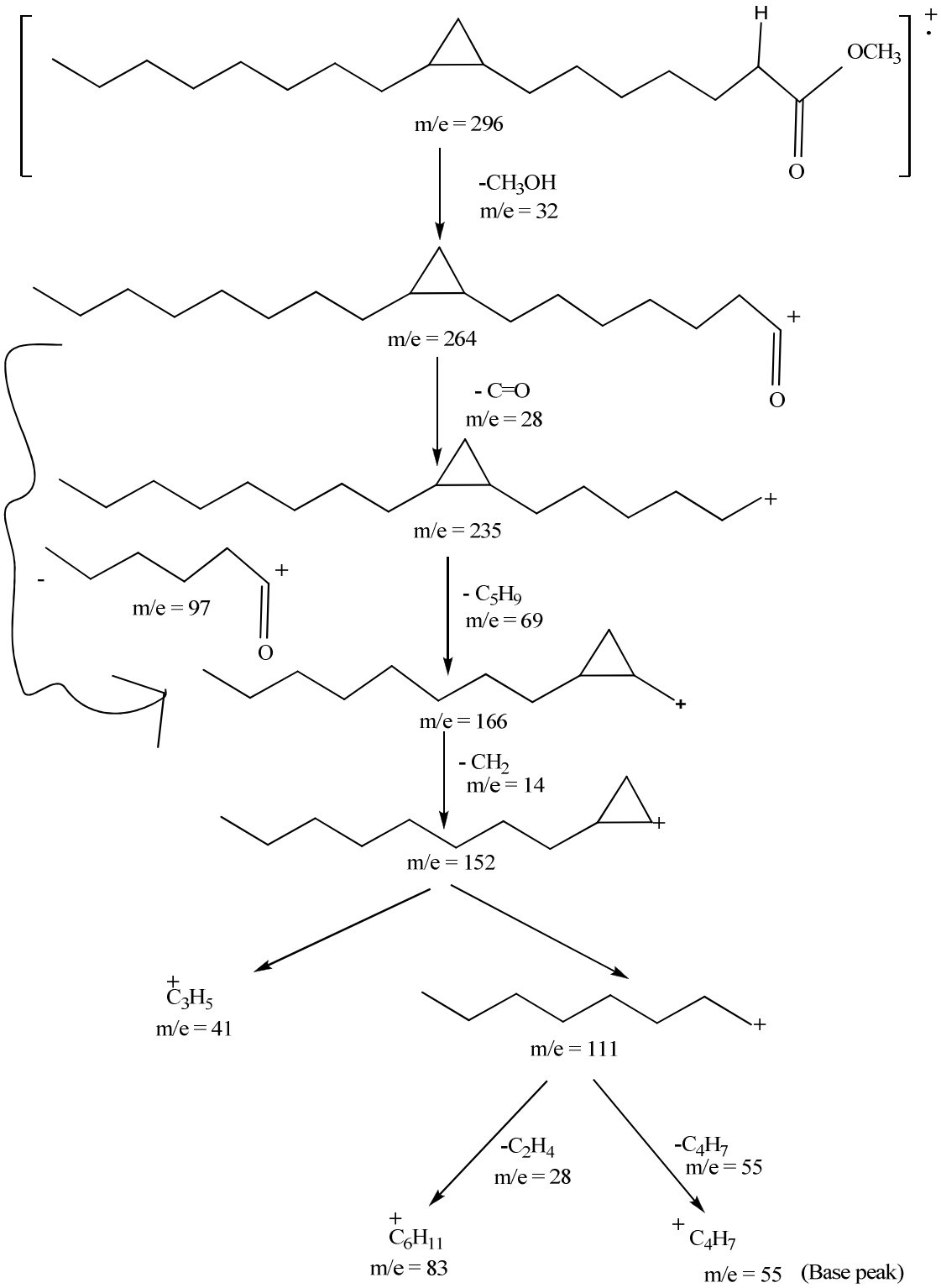
line	T_R	Area %	M^+	MR	Compounds
1.	4.985	0.19	499	-	unidentified
2.	7.495	0.47	104	$C_6H_5CH_2=CH_2$	styren
3.	7.633	0.49	85		furan,tetrahidro-2 ,2-dimethyl
4.	8.106	0.52	85	-	unidentified
5.	9.229	0.56	120	-	unidentified
6.	16.976	0.43	164	$C_6H_3OHOMeCH_2CH=CH_2$	phenol, 2-methoxy-4-(2-propenil) eugenol
7.	17.412	2.43	162	$C_6H_5CH=CHCOOMe$	2-propanoic acid, 3 phenyl methyl ester (methyl cinnamic)
8.	17.541	1.38	178	$C_6H_3(OMe)_2CH_2CH=CH_2$	benzene 1.2-dimethoxy-propenil 4-2 metileugenol
9.	19.339	1.19	206	-	unidentified
10.	20.248	0.41	538	-	unidentified
11.	20.825	0.60	175	-	unidentified
12.	20.965	0.67	324	-	unidentified
13.	21.158	0.57	181	-	unidentified
14	21.247	2.91	138	-	unidentified
15.	21.567	0.70	352	-	unidentified
16.	21.690	0.51	580	-	unidentified
17.	22.080	1.32	136	-	unidentified
18.	22.301	0.72	584	-	unidentified
19.	23.642	0.52	544	-	unidentified
20.	24.047	1.90	270	$Me(CH_2)_{14}COOMe$	hexadecanoic acid methyl ester (methyl palmitic)
21.	25.037	0.19	589	-	unidentified
22.	25.942	12.06	264		methyldihydromalvalic
23.	26.142	5.84	296	-	unidentified
24.	26.596	51.04	129	-	unidentified
25.	34.834	0.87	382	$Me(CH_2)_{22}COOMe$	tetracosanoic acid methyl ester
26.	37.678	0.73	203	-	unidentified
27.	41.530	10.77	283	-	unidentified

Main component (peak 24) has the largest area 51.04% was not identified. Peak with the largest relative % area respectively that identified were peak **22, 7, 20,** 8, 25, 3, 2, 6.

Table 2 Compounds were identified of n-hexane fraction of mahkota dewa fruit

line	**T_R**	Area %	**M^+**	**MR**	**Compound**
2.	7.495	0.47	104		styrene **(1)**
3.	7.633	0.49	85		furan, tetrahidro-2,2-dimethyl **(2)**
6.	16.976	0.43	164		phenol, 2-methoxy-4-(2-propenil), eugenol **(3)**
7.	17.412	2.43	162		2-propanoic acid, 3 phenyl methyl ester (methyl cinnamic) **(4)**
8.	17.541	1.38	178		benzene 1,2-dimethoxy-propenyl 4-2 metileugenol **(5)**
20.	4.047	1.90	270		hexadecanoic acid methyl ester (methyl palmitic) **(6)**
22.	25.942	12.06	296		Methyldihydromal-valic **(7)**
25.	34.834	0.87	382		tetracosanoic acid methyl ester **(8)**

Fragmentation analysis of peak 22 (Methyl dihydromalvalic)



Identification of Peak 22 in terms of molecular weight and pattern matching fragmentation. Based on the GC-MS spectrum of the compound has a value of SI (Similarity Index) 91% is similar to estimates from the compound in the library of reference data with MS instrument. Peak with retention time 25.49 min and area 12.06 % was estimated as methyl dihydromalvalic.

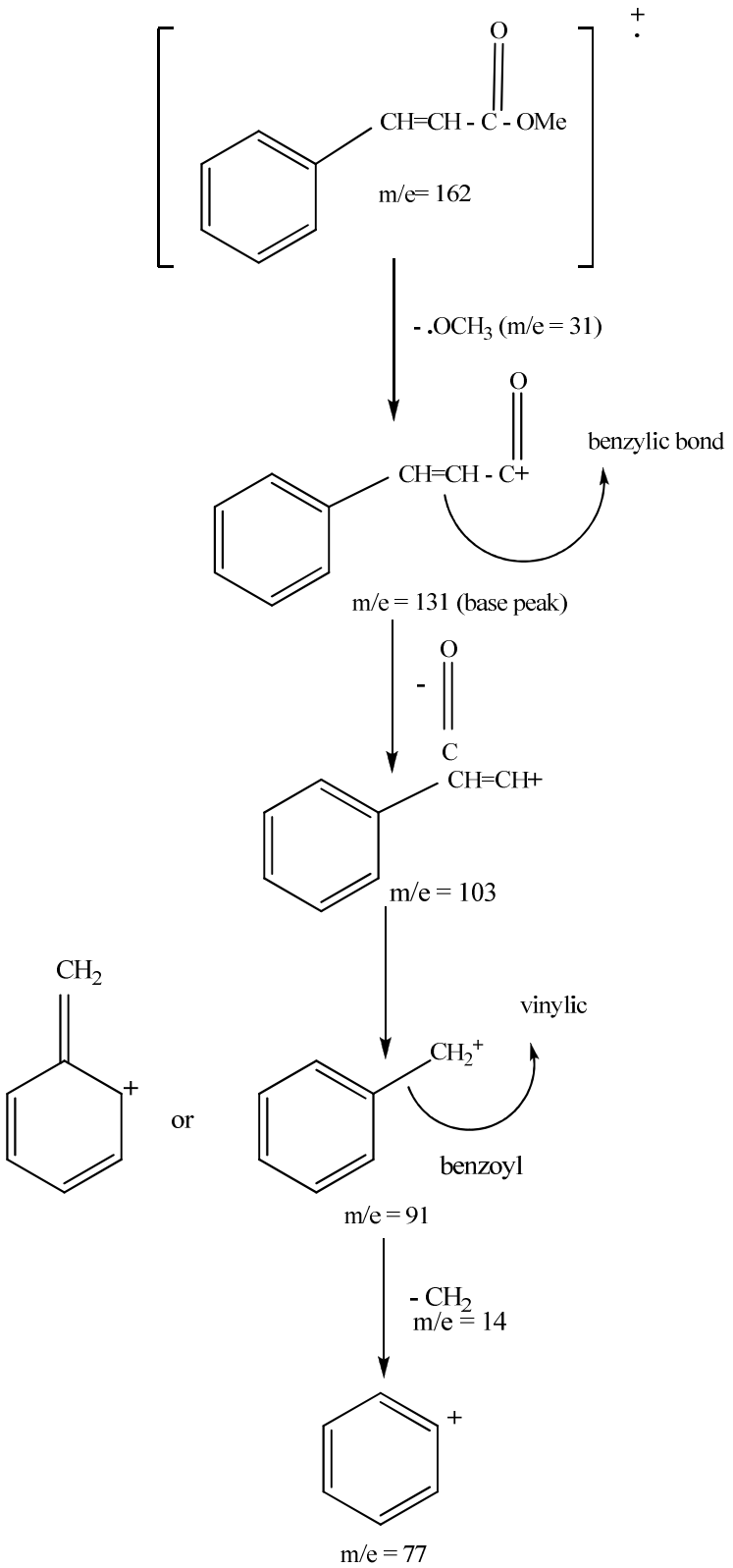
In mass spectrometry, molecules in a bomb with 70 eV electron energy, one electron apart from the molecule and formed a high-energy radical cations which have permitted big events to be fragmented is to release excess energy. Electrons will be removed from the section / molecule of the easiest places ionization, eg from electron pairs such as O or quiet than the double bond.

Peak m/e 296 which represents the molecular ion of the compound with molecular formula $C_{19}H_{36}O_2$ does not appear at sample spectrum and standard bank data library. This caused rate of fragmentation molecular ion very quickly, before molecular ion detected by instrument.

Furthermore hemiheterocyclic termination, ie termination of sigma bonds already ionization. CH_3OH molecules (m/e = 32) (m/e 296 - m/e 264) was released but not visible, only cation that appear on the m/e 264 are shown in the spectrum.

Cation can be further divided by splitting heterolitik produce other cations have lower m/e value i. e at 235, 166, 152, 111, 97, 83, 55 (base peak) and 41.

Fragmentation analysis of peak 7 (2-propanoic acid 3-phenyl methyl ester)



Fragmentation analysis of peak 7, 3 phenyl methyl ester 2 propanoic acid (methyl cinnamic)

Identification of Peak 7 in terms of molecular weight and pattern matching fragmentation. From the GC-MS spectrum of the compound has a value of SI (Similarity Index) 96% is similar to estimates from the compound in the library of reference data with MS instruments. Peak with retention time 17.412 minutes and area 2.43 % estimated as 2 propenoic acid 3 phenylmethyl ester with methyl cinnamic trivial name.

In mass spectrometry, molecules in a bomb with 70 eV electron energy, one electron apart from the molecule and formed a high-energy radical cations which have permitted big events to be fragmented is to release excess energy. Electrons will be removed from the section / molecule of the easiest places ionization, e.g. from electron pairs such as O or quiet than the double bond.

Peak at m/e 162 represents the molecular ion of the compound with molecular formula $C_{10}H_{10}O_2$

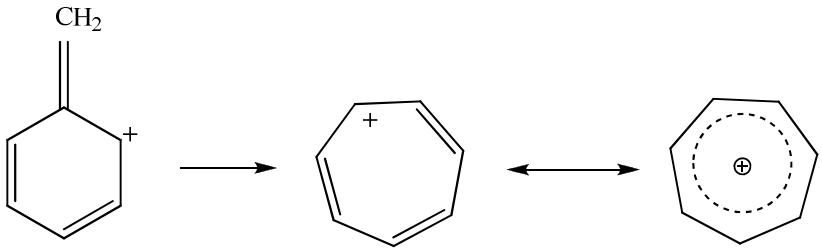
Furthermore hemiheterocyclic termination, i.e. termination of sigma bonds already ionization.

OCH_3 (m/e= 31) radical was released but not visible, only cations are shown in the spectrum.

Cation can be further divided by splitting heterolytic produce other cations have lower m/e value i. e. at 131, 103, 91 (base peak) and 77.

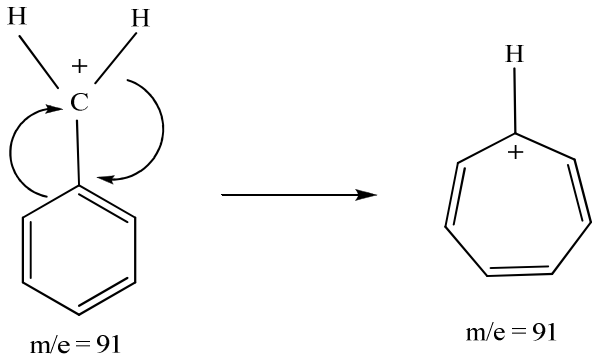
Benzylic bond termination most often found that will produce the base peak (m/e = 131).

Benzoyl cation (m/e = 91) formed can experience the expansion of the ring into tropilium ion (cation cycloheptatriena symmetric)



ion tropilium

Tropilium ion formation mechanism of usually caused by the Wagner Meerwein rearrangement of one type of resonance form cation origin.



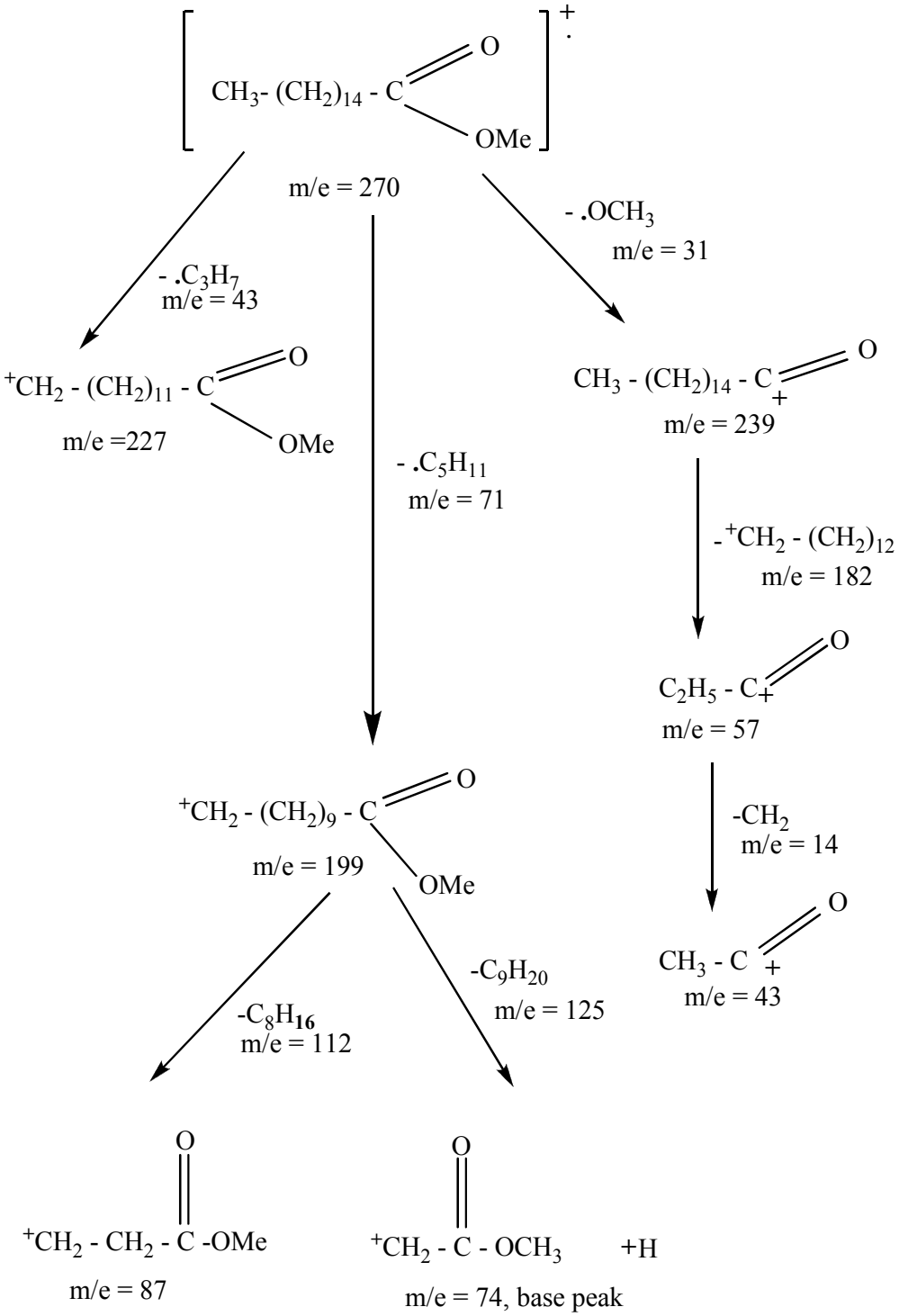
m/e = 91 m/e = 91

Termination vinylic bonding is common in aromatic systems, but likely smaller than the benzylic bond cleavage so low peak (m/e = 91)

Aromatic nucleus is a good place to establish the charge of radical cation thus generated high peak (m/e = 77)

(Cresswell, 2005)

Fragmentation analysis of peak 20, hexadecanoic acid methyl ester (methyl palmitic)



Base peak: to produce a relatively abundant fragment ions.

Identification of Peak 20 in terms of molecular weight and pattern matching fragmentation. Based on the GC-MS spectrum of the compound has a value of SI (Similarity Index) 96% is similar to estimates from the compound in the library of reference data with MS instrument. Peak with retention time 24.05 min and area 1.9 % was estimated as hexadecanoic acid methyl ester (methyl palmitic)

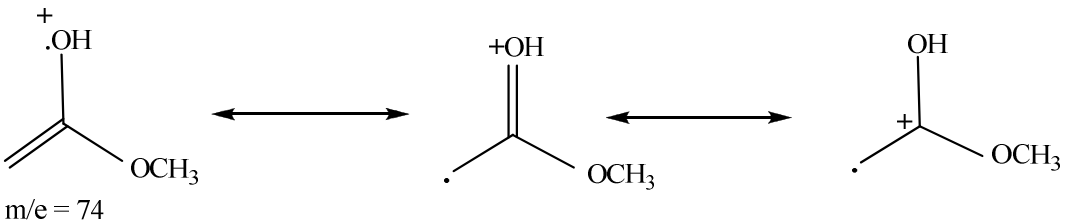
In mass spectrometry, molecules in a bomb with 70 eV electron energy, one electron apart from the molecule and formed a high-energy radical cations which have permitted big events to be fragmented is to release excess energy. Electrons will be removed from the section / molecule of the easiest places ionization, eg from electron pairs such as O or quiet than the double bond.

Peak m/e 270 which represents the molecular ion of the compound with molecular formula $C_{17}H_{34}O_2$

Furthermore hemiheterocyclic termination, i.e. termination of sigma bonds already ionization. .OCH_3 radical (m/e = 31) (m/e 270 - m/e 239) was released but not visible, only cation that appear on the m/e 239 are shown in the spectrum.

Cation can be further divided by splitting heterolitik produce other cations have lower m/e value i. e at 227, 199, 87, 74 base peak), 57 and 43

Ester mass spectrum of $CH_3(CH_2)nCOOCH_3$ (n> 2) usually have a strong ion at m/e 74 because all the esters of this type can suffer Mc Lafferty rearrangement and form the ion at m/e 74.



m/e = 74

(Cresswell, et al, 2005)

CONCLUSION

From the description above we can conclude the following things:

1. Maceration of mahkota dewa fruit (550 g) with methanol were resulted concentrated methanol extract as much as 1.2 L
2. n-hexane fraction (young yellow transparent) was obtained 3.25 g of dried, soluble in chloroform
3. GC chromatogram showed that the components of non polar fraction a successful were separated consisted of 27 components, but that can be identified only 8 peak of **22, 7, 20,** 8, 25, 3, 2, 6 (sequence based on the largest peak area)
4. Three compounds/primary component of n-hexane fraction discussed fragmentation analysis are metildihydromalvalic, 2-propanoic acid 3-phenyl methyl ester and hexadecanoic acid methyl ester (methyl palmitic)

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REFERENCES

Barker, J., 1999, *Mass Spectrometry*, 2nd ed, John Wiley and Sons, Chicester

Creswell, C. J., Runquist, O. A., Campbelll, M. M., 2005, *Spectral Analysis of Organic Compounds*, ed 10, ITB, Bandung

Fessenden, R. J., and Fessenden J. S., 1997, *Basics of Organic Chemistry Medicine*, Binarupa Aksara, Jakarta

Hakim, R. W., Nawawi, A., Adnyana, I. K., Achmad, S. A., Makmur, L., Hakim, E. H., Sjah, Y. M., Kitajima, M., 2004, Glucoside Benzophenone from Red Fruit of Mahkota

Dewa (*Phaleria macrocarpa*) and Test the Activity of DPPH and Murine Leukemia Cells P-388, *Bull. Nat. Prod.*, *Chem* (Indonesia), vol 4, pp. 67-70

Harmanto, N., 2005, the *Mahkota dewa*, Gods Heritage Drugs, ed 1, Agro Media Pustaka, Jakarta

Kitson, F. G., Larsen, B. S., and Mc Ewen, C., N., 1996, *Gas Chromatography and Spectrometry A Practical Guide*, Academic Press, San Diego

Lisdawati, V., 2007, Mahkota Dewa Fruit-Toxicity, Antioxidant Effects, and Anticancer Effect by Pharmacological Screening Tests, http://www.mahkotadewa.com/Indo/info/makalah/Vivi201002.htm.

Oshimi, S., Zaima, K., Matsuno, Y., Hirasawa, Y., Iizuka, T., Studiawan, H., Indrayanto, G., Zaini, N. C., Morita, H., 2008, Studies on the Constituents from the Fruit of *Phaleria macrocarpa*, *J. Nat. Med.* , vol 62 no 2, April 2008

Sumastuti, R. and Sonlimar M., 2006, the Cytotoxic Effect of Fruit and Leaf Extracts Mahkota Dewa (*Phaleria macrocarpa* (Scheff.) Boerl.) of Cells Hela, Pharmacology, Faculty of Medicine Gadjah Mada University, Yogyakarta, http://www.tempo.co.id/medika / arsip/122002/art-3.htm

Tambunan R. M., and Simanjuntak, P., Determination Chemical Structures of Antioxidant Glycoside Benzophenone from n-butanol Extract Fruit of Mahkota Dewa (*Phaleria macrocarpa* (Scheff) (Boerl.), 2006, *J. Pharm. Indonesia*, 17(4),184-189

Appendix 1 Photo of mahkota dewa plant

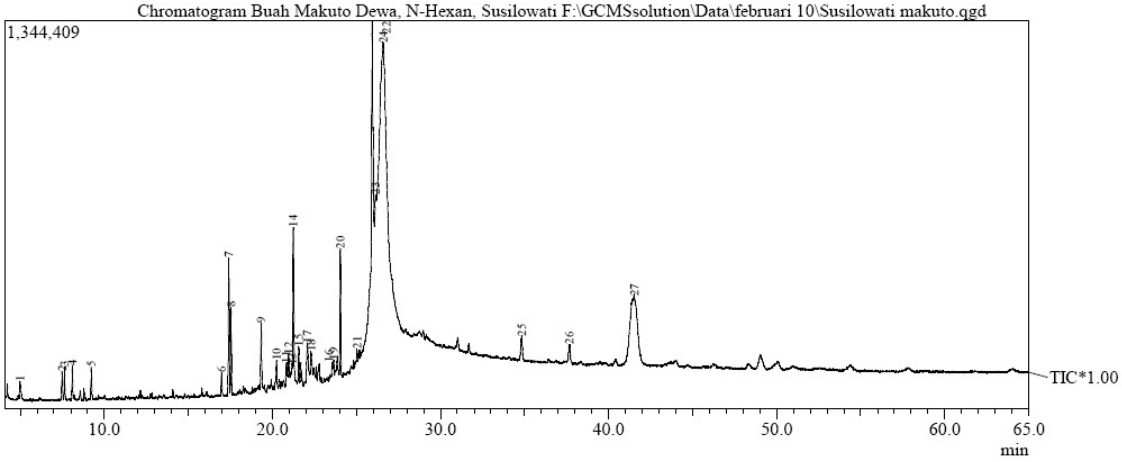
Appendix 2 GC chromatogram of the n-hexane fraction of mahkota dewa fruit

Lab.Kimia Organik FMIPA - UGM

Sample Information

Analyzed by	: Admin
Sample Name	: Buah Makuto Dewa, N-Hexan, Susilowati
Sample ID	: 130
Data File	: F:\GCMSsolution\Data\februari 10\Susilowati makuto.qgd
Method File	: C:\GCMSsolution\Data\februari 10\Susilowati.qgm
Tuning File	: C:\GCMSsolution\System\Tune1\januari 26.qgt

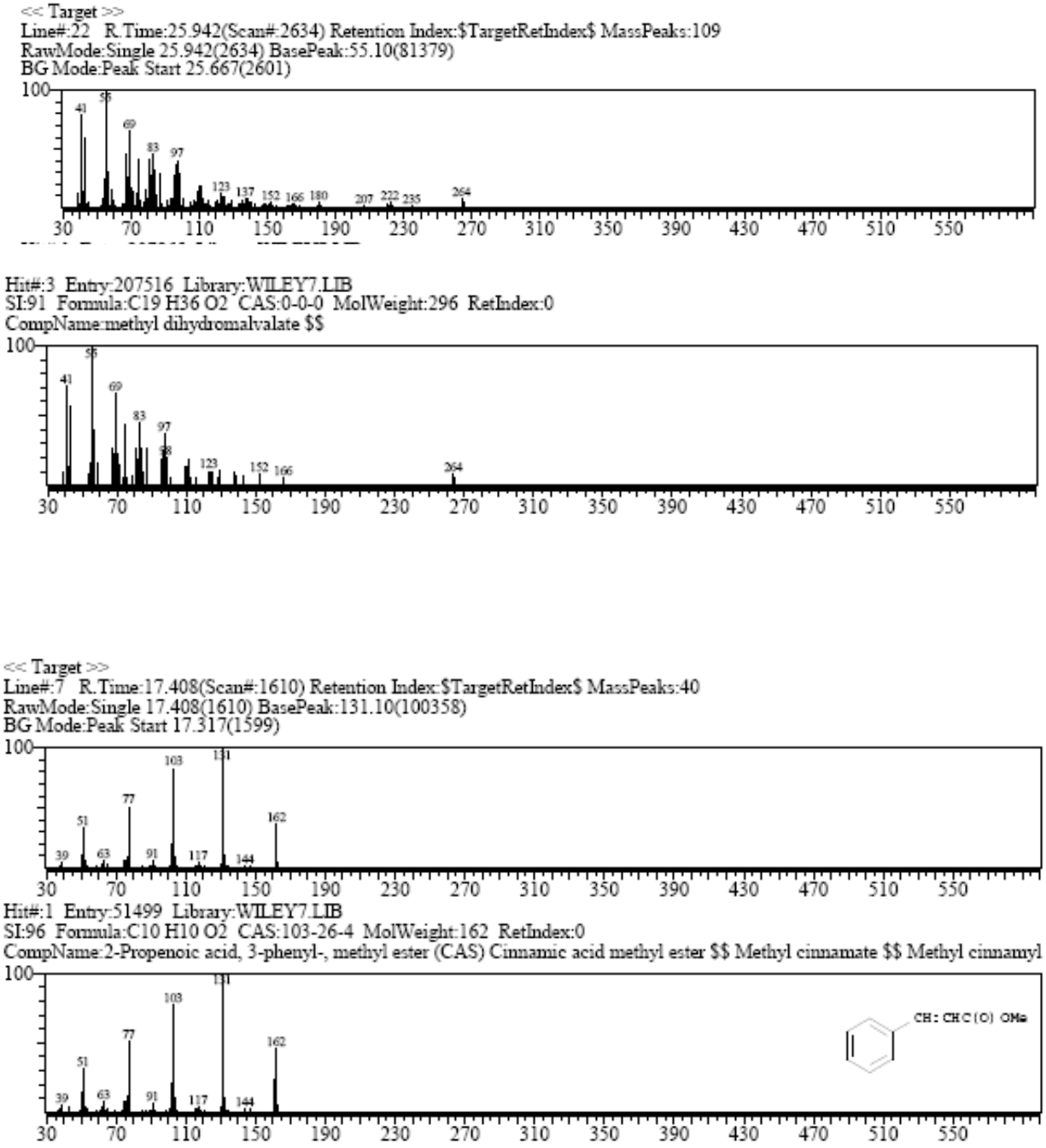
Chromatogram Buah Makuto Dewa, N-Hexan, Susilowati F:\GCMSsolution\Data\februari 10\Susilowati makuto.qgd



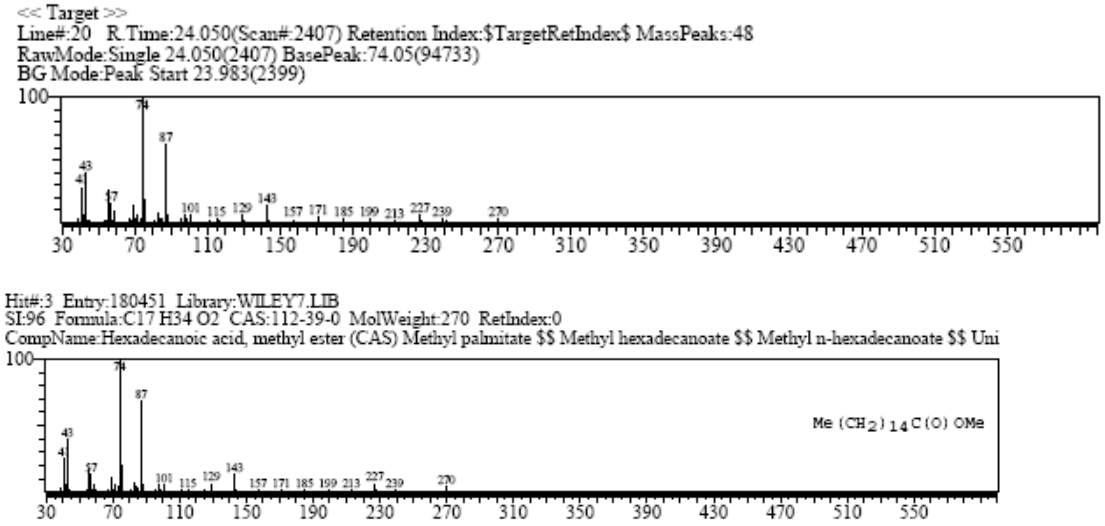
Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Name
1	4.985	4.942	5.025	126878	0.19	48205	
2	7.495	7.425	7.567	315607	0.47	97493	
3	7.633	7.575	7.708	330458	0.49	109864	
4	8.106	8.033	8.175	346973	0.52	114526	
5	9.229	9.142	9.317	376679	0.56	112794	
6	16.976	16.908	17.075	289677	0.43	80949	
7	17.412	17.317	17.483	1621598	2.43	477560	
8	17.541	17.483	17.617	919161	1.38	303300	
9	19.339	19.267	19.450	792271	1.19	229989	
10	20.248	20.175	20.342	274493	0.41	85877	
11	20.825	20.750	20.917	397650	0.60	79257	
12	20.965	20.917	21.033	448119	0.67	103766	
13	21.158	21.033	21.192	383616	0.57	60276	
14	21.247	21.192	21.375	1939653	2.91	533938	
15	21.567	21.475	21.625	468807	0.70	130392	
16	21.690	21.625	21.817	341175	0.51	76780	
17	22.080	21.958	22.217	883163	1.32	130480	
18	22.301	22.217	22.408	480609	0.72	87244	
19	23.642	23.500	23.708	350375	0.52	55894	
20	24.047	23.983	24.142	1267331	1.90	437538	
21	25.037	24.967	25.092	126574	0.19	43928	
22	25.942	25.667	26.058	8051070	12.06	1046829	
23	26.142	26.058	26.217	3900699	5.84	447572	
24	26.596	26.217	27.808	34074431	51.04	976752	
25	34.834	34.633	35.058	579933	0.87	84559	
26	37.678	37.458	37.858	488818	0.73	61388	
27	41.530	40.958	42.108	7189453	10.77	235590	
				66765271	100.00	6252740	

Appendix 3 Mass spectrum of peak 22, 7 and 20 from the n-hexane fraction of mahkota dewa compared with the data of GC-MS instrument library



Appendix 3 Continued



Appendix 4 Photo of maceration of mahkota dewa fruit with methanol by heating (60^{o}C) for 7 hours using macerator then allowed to cool at room temperature for up to 24 hours, the results of maceration of 1-4 days is reddish brown like tea.

Appendix 5 Photos of vacuum distillation equipment and Rotary evaporator for evaporation process

Appendix 6 Fractionation of methanol extract with solvents n-hexane, chloroform and ethyl acetate and photo of GC-MS instrument