AN INEXPENSIVE & SAFE METHOD FOR PREPARATION OF CARYOPHYLLENE OXIDE

RODIANAH BINTI ALIAS

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Universiti Malaysia Pahang

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ABSTRACT

Caryophyllene oxide which exists as white crystalline solids with melting points of 62 °C, is widely used as an important material in perfumery industry and recently had been patented as antitumor agent. This 99% pure oxide compound is very much more expensive than the original caryophyllene due to the difficulty in production. Experiments were carried out to produce caryophyllene oxide by using inexpensive and safe method. In this study, four objectives were studied; extraction, separation, purification of caryophyllene from clove buds which were later used in this synthesis of caryophyllene oxide in this study. In extraction, four methods were used, cleaner- ultrasonic, hydrodistillation, steam distillation and microwave to obtain clove oil and found that, hydro distillation method was found to give the highest yield of clove oil. For separation process the cayophyllene and the other non-polar molecules in clove oil were separated from eugenol, the highest yield for both clove bud and clove leaf were achieved at a ratio of clove oil: sodium hydroxide of 1: 2. Purification of caryophyllene by using vacuum distillation consist 94% component of a mixture of caryophyllene, and 5.8% alpha-cubebene by using GC/MS analyzer at 136 °C. Caryophyllene oxide was then synthesized by using four different acids, formic acid, acetic anhydride, 3-chloroperbenzoic acid, and acetic acid. The highest percentage of caryophyllene oxide formed in solution analysed by GC/MS was by formic acid (86.47%), followed by 3-chloroperbenzoic acid (81.47%), acetic anhydride (77.04%), and acetic acid (75.33%). Caryophyllene oxide was then crystallized at low temperature until subsequent analysis showed that it is 99% pure.

ABSTRAK

Kariofillin Oksida (C.O.) hadir sebagai pepejal kristal berwarna putih dengan takat lebur pada suhu 62 °C, digunakan secara meluas sebagai bahan penting dalam industri pewangi dan kebelakangan ini, C.O. dipatenkan sebagai agen antitumor. Oksida ini yang berkepekatan 99% ketulenannya adalah lebih mahal daripada kariofillin asalnya disebabkan kerumitan dalam proses pengeluaran. Beberapa eksperimen dijalankan untuk menghasilkan C.O. dengan menggunakan kaedah murah serta selamat dikendalikan. Projek penyelidikan ini merangkumi empat objektif pengajian; proses pengekstrakan, proses pengasingan, proses penulenan kariofilin daripada putik cengkih yang kemudiannya digunakan dalam sintesis kariofilin oksida dalam pengajian ini. Bagi proses pengekstrakan, empat kaedah telah digunakan iaitu ultrasonic, penyulingan dengan air, penyulingan dengan stim dan gelombang mikro untuk mendapatkan minyak cengkih, kaedah penyulingan dengan air didapati mempunyai nilai hasil minyak cengkih yang tertinggi. Dalam proses pengasingan, kariofilin dan molekul bukan polar yang lain dalam minyak cengkih telah di asingkan daripada eugenol, yield tertinggi diperolehi daripada kedua-dua putik cengkih dan daun cengkih yang dicapai oleh nisbah minyak cengkih: natrium hidroksida iaitu 1: 2. Penulenan kariofillin menggunakan penyulingan vakum mengandungi 94% komponen campuran kariofillin, dan 8% alfa-cubebene dengan menggunakan GC/MS pada 136 °C. kariofillin oksida kemudiannya disintesiskan dengan menggunakan empat jenis asid berlainan, asid formic, asetik anhidrat, asid 3-kloroperbenzoik dan asid asetik. Peratusan tertinggi kariofillin oksida yang terbentuk dalam larutan yang dianalisa oleh GC/MS adalah menggunakan asid formic (86.47%); diikuti dengan menggunakan asid 3-kloroperbenzoik (81.47%), asetik anhidrida (77.04%), dan asid asetik (75.33%). Kariofillin oksida seterusnya dikristalkan pada suhu yang rendah dan menunjukkan 99 % tulen.

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LIST OF SYMBOLS

%	Percent
^{o} C	Degrees Celsius
g	Gram
kg	Kilogram
h	Hour
MHz	Mega hertz
KHz	Kilo hertz
W	Watt
mL	Mililitre
L	Litre
min	Minute
mL/g	Mililitre per gram
β	Beta
mg	Miligram
H_2O	Water
µm	Micrometer
±	Plus minus
R	Factor (crystallography)
a	Crystallography axis
b	Crystallography axis
c	Crystallography axis

z	Crystallography axis
^{o}	Degree
w/v	Weight per volume
mm	Milimeter
m	Meter
^{o} C/min	Degree celcius per min
ml/min	Mililitre per minute
eV	Electron volt
s	Second
g/mol	Gram per mol
C.O	Caryophyllene oxide
V/V	Volume per volume
:	Ratio
cm^{-1}	Per centimeter
ppm	Part per million

LIST OF ABBREVIATIONS

GC/MS	Gas Chromatography Mass Spectrometer
RM	Ringgit Malaysia
et al.	And others
SFE	Supercritical Fluid Extraction
GC	Gas Chromatography
SFME	Supercritical Fluid Microvawe Extractor
MAHD	Microvawe assist Hydrodistillation
HSCCC	High speed counter current chromatography
ELSD	Evaporative light scatter detector
HeLa	Human cervical adenocarcinoma cells
HepG2	Human leukemia cancer cells
AGS	Human lung cancer cells
SNU-1	Human gastric cancer cell
SNU-16	Human stomach cancer
TLC	Thin layer chromatography
NEOS	Network-Enabled Optimization System
USA	United States of America
S80H	Elma model (plug-in mains supply)
He	Helium gas
N2	Nitrogen gas
H2	Hydrogen gas

DB-5	Colum phase composition
NMR	Nuclear magnetic resonance
^{1}H	Proton 1
^{13}C	Carbon 13
AM 400	Atomic mass 400
$CDCl_3$	Dichloromethane
AMU	Atomic mass unit
FTIR	Fourier transform infrared
IR	Infrared
pKa	Primary knock-on atom
C=C	Carbon double bonded to carbon
=CH	Double bonded to 1 carbon, 1 hydrogen
=CH2	Double bonded to 1 carbon, 2 hydrogen
C-H	Carbon bonded to hydrogen
C-O	Carbon bonded to oxygen

CHAPTER 1

INTRODUCTION

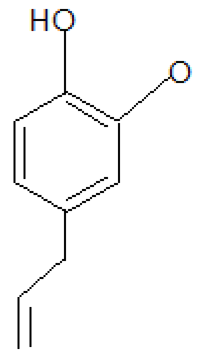
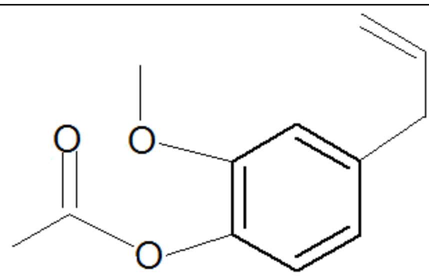
1.1 Background

Clove yields three types of crude essential oil which can be extracted from the leaves, the stems and the buds (Alma et al., 2007) namely clove leaf, clove stem and clove bud oils. The clove oil that is produced from these raw materials differs considerably in yield and quality. The yield and compositions of the oil obtained are influenced by its origin, season, variety and quality of raw material, maturity at harvest, pre- and post-distillation treatments and finally the method of distillation. Clove oil contains eugenol (Myint et al., 1996), caryophyllene as the major compositions and other minor compounds such as eugenol acetate (Huston and Li, 1991).

The best quality essential oil from the clove oil contains (80- 90%) eugenol, (15%) eugenol acetate and (5-12%) β-caryophyllene (Alma et al., 2007). Alma et al. (2007) identified 18 chemical compositions of the essential oil from Turkish Clove Buds which is produced or extracted by steam distillation method.

The analysis of clove bud oil extracted with liquid and supercritical carbon dioxide showed significant qualitative and quantitative compositional differences compared to oil obtained by the conventional hydrodistillation process. Wengqiang et al. (2007) reported clove bud oil obtained by supercritical fluid extraction (SFE) and hydrodistillation contained (53.8-55.9%) and (48.82%) percentage of eugenol respectively. The extraction of the bud flavor from the spice indicated different result by the parameters of pressure, temperature, contact time (Gopalakrishnan et al., 1990). The essential oils can be extracted by three methods hydrodistillation, microwave and ultrasonification. GC-MS analysis of the clove oils obtained by different methods showed that the composition of the clove oil was almost similar, but the relative concentration of the identified compounds was apparently different. The oil yield was influenced largely by particle size while the caryophyllene content by temperature (Wengqiang et al., 2007). Table 1.1 contains molecule structures of eugenol, eugenol acetate, caryophyllene and caryophyllene oxide and description of its properties respectively.

Table 1.1: Molecule structures and properties of eugenol, caryophyllene and its derivatives.

compounds	Molecule structure	Properties
Eugenol		Molecular formula = $C_{10}H_{12}O_2$ Molar mass = 164.20 g/mol Physical state = clear to pale yellow oily liquid Boiling point = 254 °C Soucre: Chemicalland, 2011.
Eugenol acetate		Molecular formula = $C_{12}H_{14}O_3$ Molar mass = 206.24 g/mol Physical state = clear to pale yellow oily liquid Boiling point = 281-286 °C Source: Chemicalland, 2011.

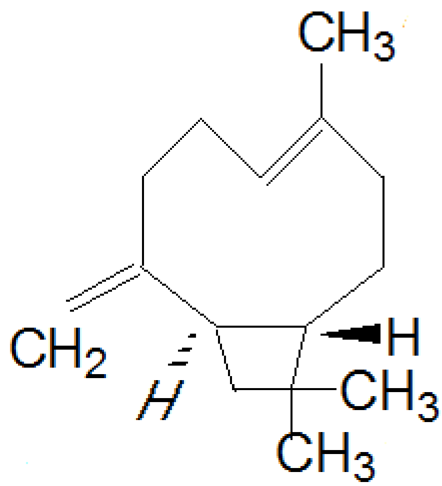
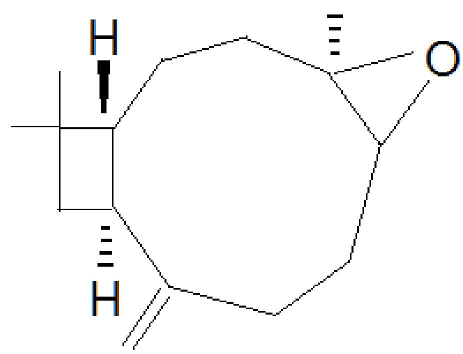
Caryophyllene		Molecular formula = $C_{15}H_{24}$ Molar mass = 204.36 g/mol Boiling point = 254 °C Category = fragrance and flavor agent Appearance = colorless clear oily liquid Odor type = spicy Source: The Good Scents Company,2012.

Table 1.1: Continued.

Caryophyllene oxide		Molecular formula = $C_{15}H_{24}O$ Molar mass = 220.36 g/mol Category = fragrance and flavor agent Appearance = white crystalline solid Odor type = woody Melting point = 60- 63 °C Source: The Good Scents Company, 2012.

Caryophyllene and their derivatives such as caryophyllene oxide, caryophyllene alcohol (Bhatia et al., 2008 and Mussinan et al., 1980) and caryophyllene acetate are widely used in flavour and fragrance compositions (Kaiser et al., 1976). In industries all caryophyllene derivatives are produced synthetically using caryophyllene as the raw material and therefore they are more expensive than caryophyllene. Some are very much more expensive because of the cost of the other materials used as well as other reasons (Kaiser et al., 1976).

Caryophyllene oxide is very much more expensive than caryophyllene itself or any other eugenol derivatives because of the difficulty in producing it. Just for comparison the price of 1 gram caryophyllene oxide of 99 % purity is RM 279.50 compared to the price of caryophyllene which is RM 10 per kilogram at the time of this work. The price of the most expensive eugenol derivative, dihydroeugenol synthesized using eugenol extracted from clove oil is only over RM 800 per kilogram. Figure 1.1 shows the molecule structure of caryophyllene and caryophyllene oxide. Table 1.2 shows the list of price of clove oil, caryophyllene and its derivatives.

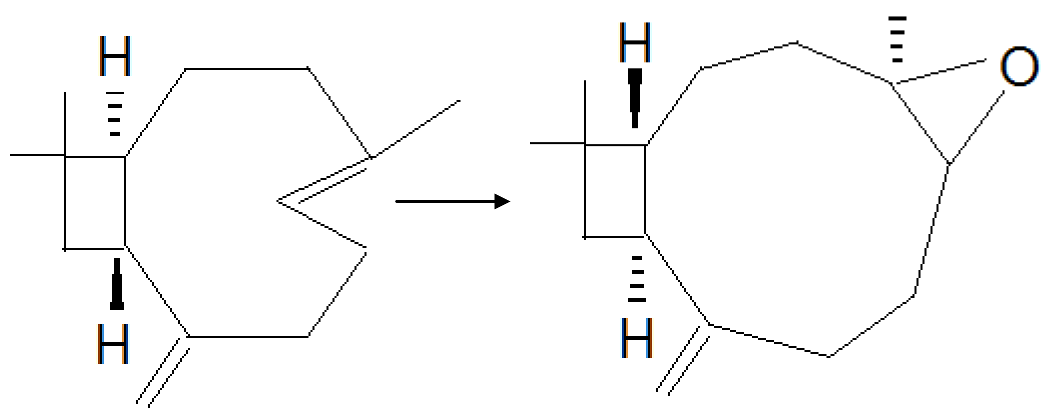


Figure 1.1: Conversion from caryophyllene to caryophyllene oxide.

Table 1.2: Prices of clove oil, caryophyllene and its derivatives.

No	Compound	Price
1	Clove oil	RM 10 / kg
2	Crude caryophyllene	RM 10 / kg
3	Caryophyllene oxide 99% pure	RM 279.50 / gram

Source: SIGMA ALDRICH MALAYSIA June 2009.

Caryophyllene oxide besides commercially applied in perfume industry (Sapra et al., 2010) and as synthetic flavoring substances (Kaiser et. al, 1976 and Yang et. al, 1999) this oxygenated terpenoid has recently been patented as antitumor agent (Choudary et. al, 2006 and Pichette et al., 2002). In previous studies, caryophyllene oxide was used as antifungal against dermatophytes (Yang et al., 1999). Other derivatives such as α-humulene and isocaryophyllene (γ-caryophyllene) are also known to have antitumor properties as shown in figure 1.2.

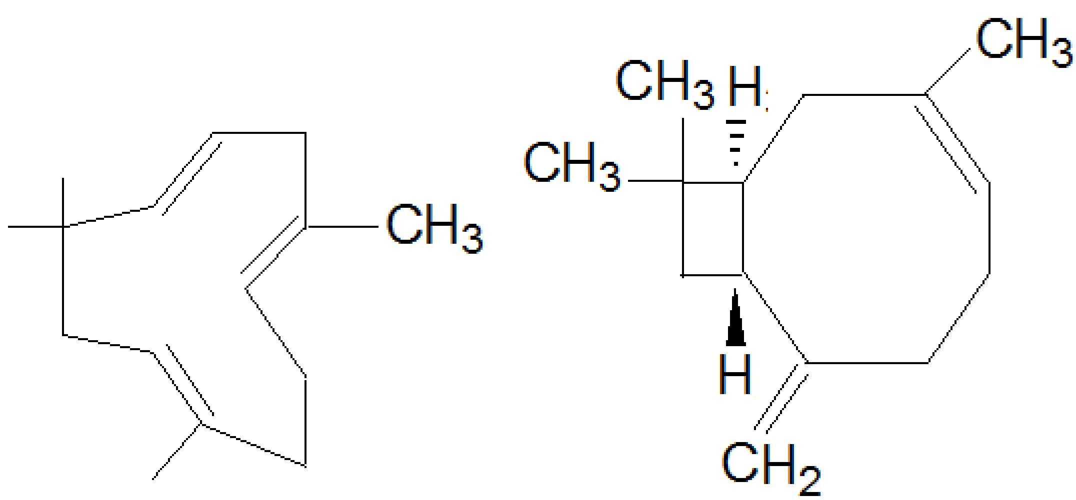


Figure 1.2: α-humulene and isocaryophyllene molecule structure.

Moreover, gluthatione-S-transferase enzyme and anticarcinogenic agent of β-caryophyllene, β-caryophyllene oxide and α-humulene have been shown to increase the activity of the detoxification which could prevent the formation of cancers (Pichette et al., 2002). Other study by Kubo et al. (1996) decribed β-caryophyllene and β-caryophyllene oxide being isolated from *Asteraceae* exhibits an antitumoral activity against solid tumor cell lines.