Synthesis Organonitrogen Compounds from Patchouli Alcohol Through Ritter Reaction with Acetonitrile and Its Toxicity to *Artemia salina* Leach.

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ABSTRACT

Patchouli oil contains a compound with biological activities to human body called the patchouli alcohol that can be further developed in medical field. This research aimed to synthesize organonitrogen compound from patchouli alcohol through Ritter reaction with acetonitrile and evaluate its toxicity towards *Artemia salina* Leach. Isolation of patchouli alcohol from patchouli oil by means of fractional distillation under reduced pressure. The synthesis of organonitrogen compound was conducted at room temperature with the mol ratio of patchouli alcohol: acetonitrile: sulfuric acid is 1:1.5:4 for 24 hours. The result showed the amount of patchouli alcohol produced from fractional distillation is 65.25%. The main product yielded through Ritter reaction is 36.93% of N-(4,8a,9,9-tetramethyl decahydro-1,6-metanonaftalen-1-il) acetamide. The starting material provided LC_{50} 77.39 ppm, meanwhile the reaction product provided higher toxicity level than that in starting material. It recorded LC_{50} value 10.39 ppm.

Key word: patchouli oil, patchouli alcohol, synthesis organonitrogen compound, Ritter reaction, toxicity

INTRODUCTION

Indonesia is one of the largest producers of patchouli oil in the world, which 90 % of the global patchouli oils was supplied from [1]. This demand opens a further exploration to find out new application, and also to increase its value such as for therapeutic application. Some studies reported the potential of patchouli oil for medicinal application. Patchouli oil showed activity by inhibiting platelet activating factor (PAF) [2], anti microbial, sedative agents, antiseptic [3], antiviral [4], and antifungal [5]. These reports encourage for further study by utilized it as pharmaceuticals precursor or starting material through molecular modification and transformation. Structure modification to form an ester derivative was reported using acetic acid and acid catalyst affording patchouli acetate [6], and dehydration reaction catalysed by H_2SO_4 was afforded patchoulenes compounds [7]. This paper report modification of patchouli alcohol to form an organonitrogen compounds, i.e a derivative-contained nitrogen with different bonding. This compounds generally correlate to biological and physiological activity for disorders of the central nervous system and its ability to interact with receptors of the body [8]. Organonitrogen compounds usually have biological activity as vasodilator, anti-inflammatory, antiviral, antimicrobial, analgesic, antidepressants,

antischistosomal, antitumor, and anticonvulsant [9]. Further studies to determine the ability of the synthesis products as a drug candidate can be evaluate through toxicity evaluation using brine shrimp lethality test (BSLT). Wijdharti *et al.* [12] stated that BSLT method is simple, rapid, inexpensive, and reliable and usually was performed as preliminary stage of the screening potential as anticancer properties before in vitro test in tumor cell.

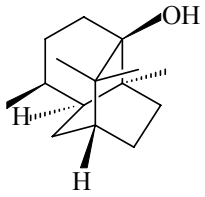

Figure 1. Patchouli alcohol structure

Structurally, it has rigid ring bonds, including tertiary alcohol group. Reaction to form an organonitrogen compound from alcohol via Ritter reaction is intermediated by a carbocation. Acidic conditions were applied if using starting material such as aliphatic or aromatic nitrile to afford N-alkyl carboxyamide [10]. Carbocation is formed in the transition state. Formation of a stable carbocation is initiated by protonation of substrate with acid, and it is further attacked by nucleophile from nitrogen compound such as acetonitrile.

EXPERIMENTAL SECTION

Materials

The chemical used has high purity grade from Merck and used as received (or as mentioned) including Na_2SO_4 anhydrous, CH_3CN, Na_2CO_3, H_2SO_4 (95-97%), diethyl eter, and DMSO. The eggs of shrimp *Artemia salina* was purchased from Biological Laboratory of Islamic State University of Malang, and patchouli alcohol (65.25% purity) was purchased from traditional farmer in Blitar, East Java, Indonesia, and was further purified using fractional distillation under reduced pressure according to procedure reported by Hapsari [8] and Novitasari [9].

Instrumentation

Spectrophotometer FT-IR (Shimadzu FTIR-8400S) and analysis in thin film using NaCl plate, Gas Chromatography-Mass Spectrometer (GCMS-QP2010S Shimadzu).

Procedure for Isolation Patchouli Alcohol from Patchouli Oil

Patchouli oil (75 mL, prior analysis with GC-MS) was dried with Na_2SO_4 anhydrous, added to a 100 mL-distillation flask and put in a series distillation apparatus with condenser column (60 cm of lenght) and vigreux column (30 cm). The process is then performed by fractional distillation under reduced pressure, the residue was obtained then analyzed with GC-MS and FT-IR

Synthesis of Patchouli Acetamide Compound Through Ritter Reaction

A number of 28.2 mL of (contain 0.1 mol patchouli alcohol) is inserted into the flask 100 mL three-neck equipped with a thermometer. A number of 7.8 mL of acetonitrile (0.15 mol) is added to the flask. The mixture was cooled to 0 °C, cold condition are maintained with the addition of salt around the flask. A number of 22 mL of 97% sulfuric acid (0.4 mol)

was added slowly to the mixture, and the mixture was left at room temperature with stirring for 24 hour. The reaction mixture was poured into an erlenmeyer containing 100 mL of cold distilled water, then was added 100 mL of diethyl eter, stirred, and separated using separating funnel. The obtained organic phase was neutralized with sodium carbonate solution, and the organic phase was further dried with Na_2SO_4 anhydrous, and filtered. The product was evaporated and further concentrated by flowing with nitrogen gas. The synthesized products were analyzed with FT-IR and GC-MS.

Toxicity Test to *Artemia salina* Leach

Artemia salina eggs were incubated in brine at pH 7-8 (48 h). Series solutions of tested substances at varying concentrations were prepared in DMSO solvent. A determined number of shrimp larvae were introduced into each solution, and were added tested samples, except for control solution without addition sample, and these were left for 24 hour. Number of life larvae was counted under microscope for each each solution used, and to evaluate the toxicity of the sample solution. Tests were carried out in triplicate.

Percent of lethality shrimp *Artemia salina* was calculated at each concentration by the equation:

$$\% = \frac{Nt}{No} X\ 100\%$$

where Nt is the number of shrimp larvae that died after incubation for 24 hours and No is the total number of shrimp larvae. LC_{50} value is then determined by linear regression on analysis of the similarities between the percent of deaths as the *y*-axis and the concentration as the *x* axis [13].

RESULT AND DISCUSSION

Isolation Patchouli Alcohol from Patchouli Oil

The yield of patchouli alcohol was obtained from fractional distillation under reduced pressure 65.25 % at 100 mmHg.

Table 1. The data content of patchouli alcohol

Parameters	Before Distillation	After Distillation
Colour of Oil	Brownish yellow	Brown
Content of GC-MS analysis	16.91 %	65.25%

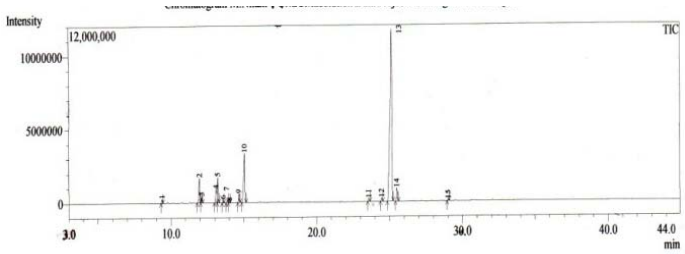

Figure 2. Chromatogram of patchouli alcohol was obtained from fractional distillation

Synthesis Patchouli Acetamide via Ritter Reaction

Product analysis using GC-MS (column Rtx-wax) was afforded chromatogram in Figure 3. Chromatogram compounds synthesized showed a peak that has 14% area of more than 1% and 12 peaks that have $M^+ = 263$ with a retention time sequence, shown in Table 2. Peak with $M^+ = 263$ major expected outcome is a product synthesis, namely N-(4,8a,9,9-tetramethyldecahydro-6,1-1-yl methanonaphtalena) acetamide, or better known as patchouli acetamide.

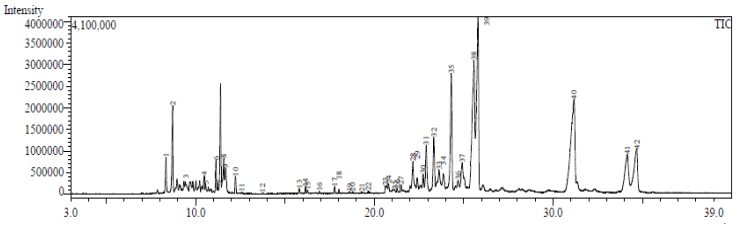

Figure 3. The chromatogram compound synthesis

Table 2. Data tabulation of mass per charge (m/z) detected from synthesized compounds

Peak	Retention Time (minute)	% Area	m/z
28	22,150	1,37%	30, 41, 43, 67, 79, 91, 110, 121, 139, 152, 161, 181, 189, 207, 248, 263
29	22,400	0,55%	30, 41, 43, 60, 70, 91, 107, 121, 135, 149, 163, 189, 206, 263
30	22,733	0,49%	30, 41, 43, 60, 79, 91, 98, 120, 137, 147, 163, 191, 206, 265
31	22,900	1,61%	30, 41, 43, 56, 81, 91, 107, 122, 136, 148, 163, 175, 189, 204, 263
32	23,325	1,71%	41, 43, 67, 91, 107, 121, 136, 148, 161, 175, 189, 204, 248, 263
33	23,625	0,81%	30, 41, 43, 67, 79, 91, 107, 120, 134, 149, 161, 189, 204, 263
34	23,867	1,38%	30, 41, 43, 67, 81, 91, 105, 124, 132, 152, 161, 178, 190, 206, 220, 248, 263
35	24,308	9,32%	30, 41, 43, 67, 79, 91, 107, 121, 133, 147, 161, 177, 189, 204, 220, 263
36	24,692	0,38%	30, 41, 43, 67, 84, 107, 122, 136, 148, 161, 189, 204, 263
37	24,917	0,99%	30, 41, 43, 67, 79, 91, 107, 120, 133, 148, 163, 177, 189, 204, 263
38	25,567	7,98%	30, 41, 43, 60, 81, 95, 114, 129, 151, 163, 177, 191, 206, 222, 250, 265
39	25,800	34,31%	30, 41, 43, 67, 79, 91, 107, 121, 133, 147, 161, 177, 189, 204, 220, 248, 263

Mass spectrum at peak 39 which has 5 area, 34,31% showed a peak of m/z = 30,41,43, 67, 79, 91, 107, 121, 133, 147, 161, 177, 189, 204, 220, 258, and 263 (Figure 4). Presence of

the carbonyl group in patchouli acetamide compound results cleavage α, peak m/z = 263 release CH_3 so that produce peak m/z = M^+ -15 = 248, then the release CO to form peak of m/z = 220. Cleavage α to C-carbonyl produces a peak m/z = M^+ - 220 = 43. Peak m/z = M^+ - 59 = 204 is obtained from the release NH_2COCH_3, then the release CH_3 to form the peak m/z = M^+ - 74 = 189. Peak m/z = 220 of the release of C_3H_7 (M^+ - 43) then release NH_2COCH_3 thus forming the peak m/z = 161. Peak m/z = 161 have thus generated the release of C_4H_8 peak m/z = 91. Peak m/z = 91 release C_2H_2 respectively so as to produce the peak m/z = 41. Suggested fragmentation pattern for compound patchouli acetamide shown in Figure 6.

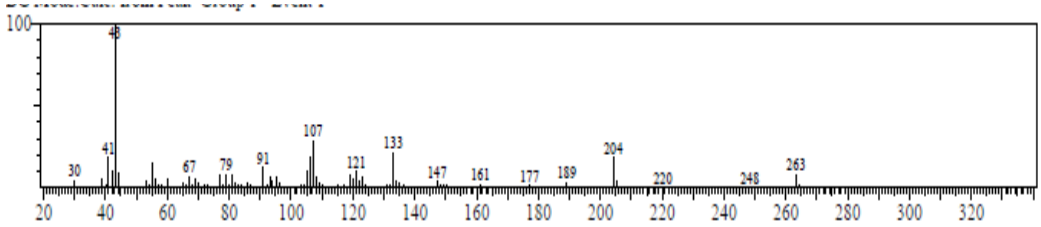

Figure 4. The Mass Spectrum Peak 39 with Retention Time 25,8 minutes.

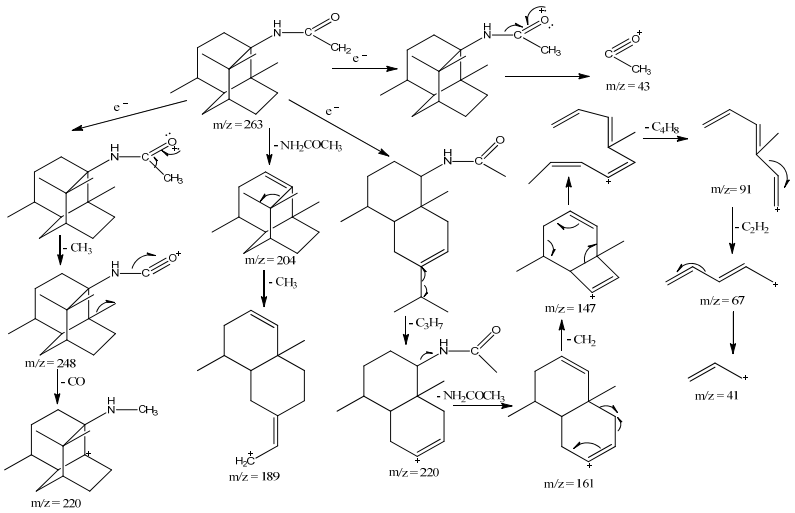

Figure 5. Suggested fragmentation for patchouli acetamide

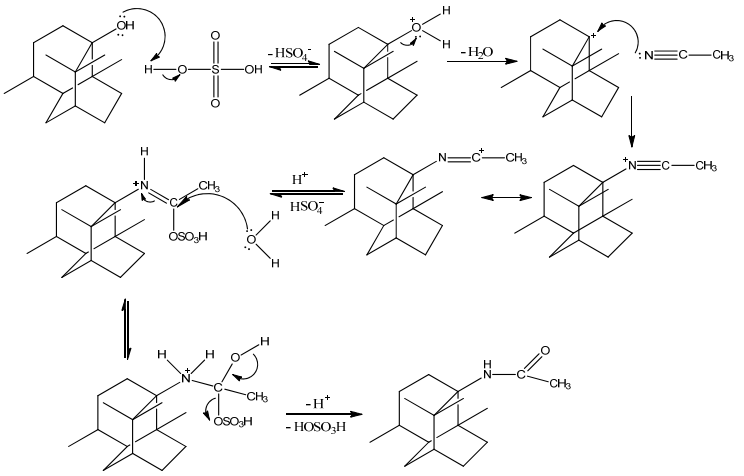

Figure 6. Plausible mechanism reaction the formation of patchouli acetamide

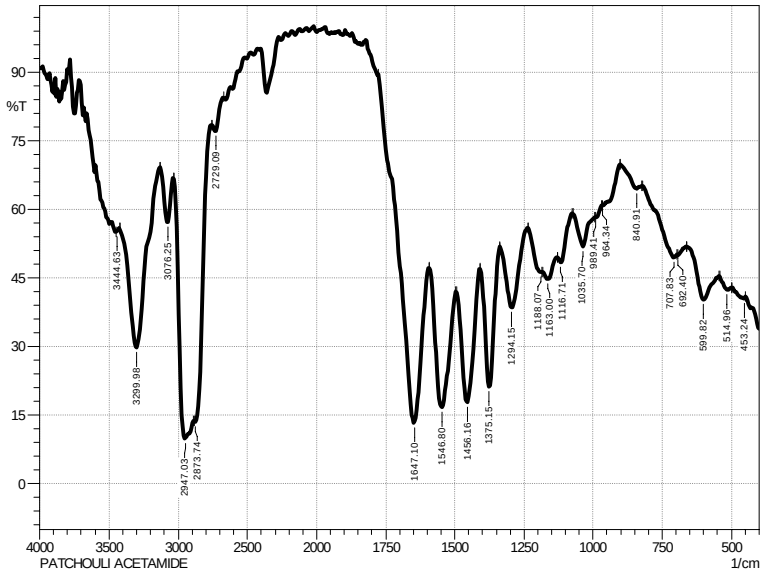

Figure 7. Infrared spectrum of product synthesized

Table 3. Data functional group product synthesis

Wavenumber (cm^{-1})	Type of vibration
3299, 98	Stretching N-H
2947,03	Stretching C-H
1647,10	Stretching C=O in amide
1546,80	Bending N-H
1456,16	Bending C-H in alkene

Based on FT-IR spectrum of compounds synthesized (patchouli acetamide) and functional groups of data (Table 3), show a strong absorption at wavenumber 3299 cm^{-1} which is the N-H stretching vibration of secondary amide. It was also supported by absorption at 1647,10 cm^{-1} for vibration of the amide carbonyl stretching and at 1546,80 cm^{-1} for N-H bending. This indicates that the presence of secondary amide compounds contained in the compounds synthesized.

Toxicity Test to *Artemia salina* Leach.

Product synthesis containing patchouli acetamide 36.93% gives higher toxicity. Structure of patchouli acetamide has a lone pair on nitrogen atom, and also carbonyl group has two lone pair on oxygen. These functional groups are predicted able to increase interaction by formation of hydrogen bonds with DNA, and distract the metabolism of *A. salina* Leach.

Table 4. The calculation result for LC_{50} from BSLT test

No.	Sampel	Graph Equation	LC_{50}
1	Starting material	Y = 0,6462x R^2 = 0,9809	77,38 ppm
2	Product Synthesis	Y = 4,575x R^2 = 0,983	10,93 ppm

CONCLUSION

It can be concluded that the main products of the synthesis of patchouli alcohol with acetonitrile trough Ritter reaction is N-(4,81,9,9-tetramethyl decahydro-6-1-1-yl methanonaphtalene) acetamide or patchouli acetamide (36.93 % yield). The synthesized compounds have a toxicity level greater than starting material, (10.93 ppm), and it is potential for lead drug compounds.

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