SYNTHETIC STUDY OF Pc-2, AN ACTIVE COMPOUND FROM INDOONESIAN RED BETEL LEAF

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ABSTRACT
PC-2 is one of the active compounds isolated from Indonesian red betel leaf. The structure of the compound is named 2-allyl-4-(1'-hydroxy-1'-(3",4",5"-trimethoxyphenyl)propan-2'-yl)-3,5-dimethoxycyclohexa-3,5-dienone and included in the neolignan’s structure category. This compound was reported to have very good biological activity in giving antiproliferative effects on human breast (T47D) cells. From 2.12 g of its leaf methanolic extract, only 12.1 mg of Pc-2 was collected. This amount is too small to do more studies about its biological activities. The synthetic work should be applied to get a sufficient amount. This paper is aimed to explain the synthetic study and design of the Pc-2 compound. The first approach is by involving the aldol reaction in the synthetic pathway through the disconnection approach. The result showed that this attempt did not work very well. The second approach is to design the new synthetic pathway by using the synthia Organic Retrosynthesis Software by Merck. This software gave a reasonable result to be tried. The result proposed the use of the Suzuki coupling reaction for the new synthetic pathway of Pc-2.

Keywords: Active Compound, Disconnection Approach, Indonesian Red Betel Leaf, PC-2, Synthia Retrosynthesis Software, Synthetic Study

INTRODUCTION
Many plants have been reported to have various kinds of medicinal activities in different traditional medicines in Indonesia. Some of that consist of plants from the genus Piper. The study about the potency of Betel leaves extracts as antibiofilm has been reported against Methicilin Resistant Staphylococcus aureus (MSRA).¹ In Indonesia, one of the genus Piper plants is Red betel (Piper crocatum Ruiz & Pav). This species has red silvery leaves. It is used to use as a medicinal plant for treating various diseases. Its scientific approval also has been investigated and published in many publications around the world.² A bioactive compound from red betel leaves was studied in silico for its activity as an anticancer agent against colon cancer marker proteins.³ Antibacterial activity of the phytochemical compound of this red betel leaf has also been studied against bacteria in dental caries.⁴ The methanolic extract of this Red betel (Piper crocatum Ruiz & Pav) was reported as antiproliferative effect on human breast (T47D) cells.⁵ The active compound from Red betel also has been isolated from this methanolic extract. It was found that the active compound showed an immunomodulatory effect and histopathological features of the mouse liver and kidney. This isolated compound was then named PC-2. Pc-2 can increase macrophage phagocytosis as well as nitric oxide production but not lymphocyte proliferation. The compound was code as Pc-2 where its chemical name is 2-allyl-4-(1'-hydroxy-1'-(3",4",5"-trimethoxyphenyl)propan-2'-yl)-3,5-dimethoxycyclohexa-3, 5-dienone. 2.12 g of this red betel leaf methanolic extract yielded 12.1 mg of Pc-2 only. The structure of Pc-2 is shown below.⁶ This is a neolignan-classified structure compound.⁷ This report is a model study of the synthesis of Pc-2. The Synthetic design of this compound is by cleavage of the bond in the middle that connects both aromatic rings. The band has hydroxyl and methyl groups attached.

Synthon-1 and synthon-2 could be combined through the aldol reaction. This paper explains the study of the main reaction that might be involved in the synthesis of Pc-2. The process will start with the compound-like of synthon-1. Only synthon-1 is being focused on. The general synthetic equivalent of synthon-1 is an
aldehyde. So, it can be started by using the below disconnection approach as a model. This study only reports the model synthesis of the aldehyde compound of synthon-1.

![Structure of Pc-2](image)

**Fig.-1: Structure of Pc-2**

**Scheme-1: Cleavage Between Both Aromatic Rings on Pc-2**

![Scheme](image)

**Scheme-2: Disconnection Approach of Compound 8**

This research is aimed to synthesize the Pc-2 compound and study the possibility of the synthetic pathway of that compound.

**EXPERIMENTAL**

**Synthesis of 2-Prop-2-ynyloxy-tetrahydropyran, 2**

To a round bottom flask (2L), propargyl alcohol (10 g; 178 mmol; 1 eq) was added into DCM (900 mL) at 0 °C. Then DHP (40.6 mL; 446 mmol; 2.5 eq) and TsOH (3.066 g; 17.8 mmol; 10 mol%) were added respectively. The color of the reaction mixture was changed from transparent brown to black. The reaction mixture was left for 1.5 h for completion. Then it was quenched with NaHCO$_3$ (aq), extracted with CHCl$_3$ (2 X 200 mL) and the organic phase was evaporated under vacuum. The product was isolated by using flash column chromatography (EtOAc: Hexane = 1:9), yielded a colorless oil in 80 %, Rf = 0.36 (EtOAc: Hexane = 1:9); $^1$H-NMR (CDCl$_3$, 400 MHz) $\delta$ 1.45-1.55 (1H, m, 3-CH$_2$), 1.55-1.61 (1H, m, 3-CH$_2$), 1.67-1.76 (2H, 1H-NMR (CDCl$_3$, 400 MHz) $\delta$ 1.45-1.55 (1H, m, 3-CH$_2$), 1.55-1.61 (1H, m, 3-CH$_2$), 1.67-1.76 (2H,
m, 2-CH₂), 1.78-1.83 (2H, m, 1-CH₂), 2.389 (1 H, s, H-C≡), 2.38-3.51, (1H, m, 4-CH₂), 3.72-3.86 (1H, m, 4-CH₂), 4.16-4.28 (2H, m, 6-CH₂), 4.55 (1H, s, 7-H).

**Synthesis of Methyl-diphenyl-[3-(tetrahydro-pyran-2-yloxy)-prop-1-ynyl]-silane, 3**

To neck round bottom flask (100 mL), Propargyl-OTHP (7.13 mmol; 1 g) was added to THF (18 mL) at -78 °C and followed by adding of n-BuLi (8.556 mmol; 5.889 mL). After 1 h, the reaction was cooled to -78 °C and Diphenyl-methyl-silane chloride (8.556 mmol; 1.8 mL) was added to the mixture. The yellowish transparent color was changed into a colorless solution in 1 h to reach r.t. The reaction mixture was quenched with buffer (10 mL), extracted with EtOAc (3 x 30 mL), and washed with water (2 x 10 mL) and brine (1 x 10 mL). The organic phase was dried over Na₂SO₄, filtered, and evaporated under a vacuum. The product was isolated by using flask column chromatography (EtOAc: Hexane = 1: 15) yielded a colorless oil in 44 %, Rf = 0.36 (EtOAc: Hexane = 1: 15); ¹H-NMR (CDCl₃, 400 MHz) δ 0.7 (3H, s, -CH₃), 1.53-1.68 (4H, m, 3-H and 2-H), 1.71-1.87 (2H, m, 1-H), 3.50-3.55 (2H, m, 4-H), 3.83-3.89 (1H, m, 4-H), 4.37 (2H, s, 5-CH₂), 7.34-7.43 (6H, m, Ph), 7.63-7.65 (4H, m Ph); ¹³C-NMR (CDCl₃, 100 MHz) 19.20, 25.51, 30.42, 55.02, 62.21, 96.99, 128.07, 129.85, 134.66; IR (neat): νmax 2942, 1428, 1117, 1027, 792, 728 cm⁻¹; HRMS (ESI): [M+Na]⁺ calculated for [C₂₁H₂₄NaO₂Si]⁺: 359.1443, foun: 359.1443

**Synthesis of 3-(Methyl-diphenyl-silanyl)-prop-2-yn-1-olcohol, 4**

To dimethyl-phenyl-OTHP (4.62 g; 13.73 mmol) in two necks round bottom flask (100 mL), was added amberlyst-15 (20 wt %, 924 mg) and MeOH (28 mL, 0.5 M). The reaction mixture was refluxed for 1.5 h. Product 4 was isolated by flash column chromatography (EtOAc: Hexane = 1: 9, gradually changed to EtOAc: Hexane = 1: 5 ) and yielded a yellowish-colorless oil in 91 %, Rf = 0.09 (EtOAc: Hexane = 1: 9); ¹H-NMR (CDCl₃, 400 MHz) δ 0.73 (3H, s, CH₃), 1.77 (1H, s, OH), 4.36 (2H, s, CH₂), 7.37-7.45 (6H, m, Ph), 7.62-7.67 (4H, m, Ph); ¹³C-NMR (CDCl₃, 100 MHz) IR (neat): vmax 3336, 3068, 2176, 1588, 1428, 1114, 1040, 984, 793 cm⁻¹; HRMS (ESI) : [M+Na]⁺ calculated for [C₁₆H₁₄NaOSi]⁺: 275.0868, foun,: 275.0868

**Synthesis of 3-(Methyl-diphenyl-silanyl)-prop-2-yn-1-olcohol, 5**

To dimethyl-phenyl-propargyl-OH (2.5 g; 9.91 mmol) in two necks round bottom flask (100 mL), was added MnO₂ (20 eq. 17,136 mg) and DCM (40 mL, 0.25 M). The reaction mixture was left for 3 h at room temperature. After filtration and solvent evaporated, product 5 was isolated by using flash column chromatography (EtOAc: Hexane = 1: 9) yielded a colorless oil in 18 %, Rf = 0.41 (EtOAc: Hexane = 1: 9); ¹H-NMR (CDCl₃, 400 MHz) δ 0.82 (3H, s, CH₃), 7.48-7.64 (6H, m, Ph), 7.66-7.663 (4H, m, Ph), 9.26 (1H, s, CHO); ¹³C-NMR (CDCl₃, 100 MHz); IR (neat): vmax 3070, 2153, 1666, 1429, 1387, 1116, 997, 795, 731, 697 cm⁻¹; HRMS (ESI) : [M+Na]⁺ calculated for [C₁₆H₁₄NaOSi]⁺: 273.0709.

**RESULTS AND DISCUSSION**

The whole process of reaction can be seen in schemes 3-7. Propargyl alcohol was transformed into protected propargyl alcohol and then removed from the protecting group to the alcohol group again. This alcohol group was next to be oxidized into an aldehyde.
Starting with the protection of propargyl alcohol, the given condition yielded compound 2 in 80%. The reaction mechanism is as in Scheme-3. In the beginning, 3,4-DiHydro-2H-pyran (DHP) was protonated by p-Toluenesulfonic acid (TsOH•H2O) resulting in a positive charge on the oxygen of DHP. This species was attacked by Propargyl alcohol which resulted in protected propargyl alcohol.

\[ \text{DHP} \rightarrow \text{DHP}^+ \]

1. n-BuLi (1.2 eq) THF (0.4 M) -78 °C - rt, 1 h
2. Ph₂MeSiCl (1.2 eq) -78 °C - 0 °C, 1 h
3. Amberlyst-15 (1.2 eq) MeOH (0.5 M) reflux, 1 h
4. MnO₂ (20 eq) DCM (0.25 M) rt, 3 days

Scheme-3: Synthesis of Aldehyde 5

Sodium bicarbonate was to quench p-Toluenesulfonic acid. The next reaction is the formation of 3 by reacting compound 2 with chloromethyl diphenyl silane. After one hour, the reaction was stopped and the product was tried to be isolated, which was resulting in failing to be isolated. Buffer was to quench n-butyl lithium. Compound 3 can be successfully isolated in 44% pure isolated compound. The reaction mechanism is as follows where nBuLi acted as the catalyst.

\[ \text{OTHP} \quad 2 \quad \text{from Amberlyst-15} \]

Scheme-5: Reaction Mechanism for the Formation of Compound 3

Compound 3 was subjected to the next reaction, which yielded 4 in 91%. The protection reaction was carried out by amberlyst-15 for one hour.

\[ \text{OTHP} \quad 4 \quad \text{from Amberlyst-15} \]

Scheme-6: The Reaction Mechanism for the Formation of Compound 4
The reaction was continuous to the next step where alcohol was oxidized to yield compound 5 in 18%. Compound 5 was then reacted with the aldehyde and ester phosphine. So far, no aldol product was obtained even after five days of reaction. Hence, after four hours of reaction without further isolation and the ester phosphine was added. Only product 7 was observed as the main product of the Wittig reaction. The organocatalyst, (R)-diarylprolinol did not help much yet.

According to Scheme-3 and Scheme-7, the aldol product, β-methyl alcohol cannot be successfully formed. This pathway was not successful yet when applied to the synthesis of Pc-2. The other proposed synthetic pathway is initiated by the oxidation of aryl aldehydes to phenols/hydroxyheteroaryls via formats (Scheme-8). Compound 4-bromo-3,5-dimethoxy-benzaldehyde 9 is converted to 4-bromo-3,5-dimethoxyphenol 10 with H₂O₂ or mCPBA. Iodination of aromatic compounds, 4-bromo-3,5-dimethoxyphenol 10 to C₈H₈BrIO₃ 11, by I₂ or other iodinating agents e.g. NIS. Suzuki coupling of alkyl-9-BBNs with aryl iodides perform C₈H₈BrIO₃ 11 and 9-allyl-9-bora-bicyclo[3.3.1]nonane to C₁₁H₁₃BrO₃ 12 by using Pd catalyst in a basic solvent. Suzuki coupling is the palladium-catalyzed cross-coupling between organoboronic acid and halides. Recently the catalyst and the method have been broadly developed especially the use of 9-BBN. 

The next step (Scheme-9) is the synthesis of secondary amides from aldehydes, 3,4,5-trimethoxy-benzaldehyde 14 and n-methyl-methanamine 13 turn into 3,4,5-trimethoxy-benzoic acid dimethylamide 15 in CuI, MS 4A, toluene, 100 °C and air.
One pot arylative synthesis of ketones from benzamides is the further reaction where \(\text{C}_{11}\text{H}_{13}\text{BrO}_{3}\) 12 and 3,4,5-tri methoxy-benzoic acid diethylamide 15 and bromoethane 16 turn into \(\text{C}_{23}\text{H}_{28}\text{O}_{7}\) 17 by Pd[(PtBu)\(_3\)]\(_2\) as a catalyst in THF at 60 °C.\(^2\) Last reaction is the reduction of ketones with NaBH\(_4\), \(\text{C}_{23}\text{H}_{28}\text{O}_{7}\) 17 to \(\text{C}_{23}\text{H}_{30}\text{O}\) Pc-2 with NaBH\(_4\) as the catalyst in MeOH at 0 °C. This is a very common chemistry seen in basic chemistry textbooks (Scheme-10).

![Chemical structure of the target molecule, Pc-2](image)

**Scheme-10: The Synthesis of the Target Molecule, Pc-2**

**CONCLUSION**

The synthetic study of Pc-2, isolated from Indonesian Red betel leaf, has been studied in two methods of pathways. The first method was carried out in the laboratory. Throughout the whole reaction, the last step of reactions is still not working. This is the attempt to form the product of the aldol reaction. The second method is the proposed pathway by Synthia Retrosynthesis Software and seems that it will work very well.

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**CONFLICT OF INTERESTS**

The authors declare that there is no conflict of interest.

**AUTHOR CONTRIBUTIONS**

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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**REFERENCES**


[RJC-7052/2020]