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INFLUENTIAL FACTORS OF PICTET-SPENGLER REACTION IN TOTAL SYNTHESIS OF CANTHIN-6-ONE

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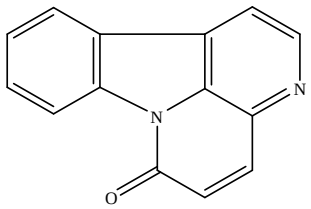
Graphical abstract



Abstract

The family of Simaroubaceae is known to contain quassinoids and canthin-6-ones, secondary metabolites that have been reported to possess numerous biological activities such as anticancer. A biomimetic total synthesis of canthin-6-one using Pictet-Spengler condensation according to the procedure by Czerwinski *et al*. (2003) was explored. The aim of this study was to determine the viability of this reaction for structural modification. In this report, influential factors of carboxyl mediated Pictet-Spengler condensation reaction would be discussed.

Keywords: Pictet-Spengler, canthin-6-one, Simaroubaceae

Abstrak

Keluarga pokok Simaroubaceae diketahui mengandungi metabolit sekunder kuasinoid dan kantin-6-on yang memiliki banyak aktiviti biologi seperti anti-kanser. Sintesis keseluruhan biomimetik kantin-6-on menggunakan kondensasi Pictet-Spengler mengikut prosedur oleh Czerwinski *et al*. (2003) telah dikaji. Tujuan kajian ialah untuk menentukan kesesuaian tindak balas ini di dalam pengubahsuaian struktur. Laporan ini membincangkan faktor-faktor yang mempengaruhi tindakbalas kondensasi mediasi karboksil Pictet-Spengler.

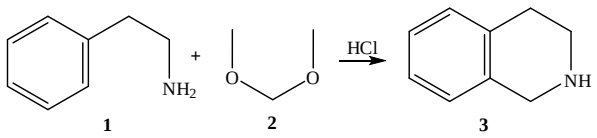
Kata kunci: Pictet-Spengler, kantin-6-on, Simaroubaceae

1.0 INTRODUCTION

Pictet-Spengler (PS) reaction is considered the most powerful method to date for the synthesis of alkaloids specifically to indole and isoquinoline. The reaction was conceived and created by Swedish chemists Amè Pictet and Theodor Spengler in 1911 [1]. They had reacted β-phenylethylamine (**1**) and formaldehyde dimethyl acetal (**2**) in the presence of hydrochloric acid (Scheme 1). The reaction undergone a condensation before a cycloaddition thus forming an alkaloid, 1,2,3,4-tetrahydroisoquinoline (THIQ) (**3**).

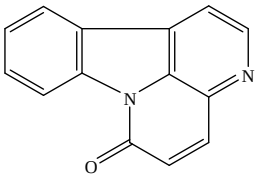
Canthin-6-one (**4**), a β-carboline indole alkaloid was first isolated by H. F. Haynes in 1951 from the tree *Pentaceras australis* Hook. F. of the family Rutaceae [2]. Almost all canthin-6-one compounds are found exclusively in Rutaceae and Simaroubaceae. However, 29 of 35 alkaloids isolated from nature were

isolated from Simaroubaceae. Their occurrence is mostly in the root bark and bark [3].



Scheme 1 Pictet-Spengler Reaction of THIQ

Canthin-6-one is currently commercialised as an antimyobacterial drug. It is used to treat or prevent infections caused by *Mycobacteria*, a genus of actinobacteria, which include pathogens responsible for causing tuberculosis and leprosy [4]. It has also been reported to possess various biological activities such as; antivirus, antifungal, anticancer, cAMP PDE inhibitory, protective effect of delayed neuron death and functional damage of the brain (Koike, 2003), anti-protozoal [6], anti-proliferative [7], sexual prowess [8], anti-inflammatory [9], [10], anti-*Trypanosoma cruzi*[11], anti-leukemic [12], leishmanicidal [13], and cytotoxicity [14].



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The total synthesis of canthin-6-one undertook in this study reflected considerations of mimicking natural synthesis in terms of its starting material and reaction, hence tryptamine (**5**) becomes the starting material and PS reaction is the key step. Decisively, Czerwinski's application of PS reaction was deemed viable in terms of facilities and material availability for structural modification work planned. The main focus of this report would be to discuss the key reactions in the synthesis of canthin-6-one strategy which would explain the yield of the products obtained.

2.0 EXPERIMENTAL

^{1}H-NMR spectra were measured in deuterated solvent on Bruker NMR 300 MHz Spectrometers with tetramethylsilane as an internal standard. Chemical shifts are expressed in δ (ppm) units downfield from TMS.

N_b-benzoyltryptamine (Nb). A stirred solution of tryptamine (5 g, 31.20 mmol), NaOH (3.12 g, 78 mmol) and anhydrous acetone (150 mL) was cooled at -30°C. Later, benzoyl chloride (4.35 mL, 37.42 mmol) was added dropwise. The mixture was allowed to stir for 12 hour. Then, the reaction mixture was quenched with water (100 mL). Additional water (500 mL) was then added and the suspension was filtered. The filtrate was extracted with diethyl ether (3 × 200 mL), dried over anhydrous K_2CO_3 and the solvent was then removed under reduced pressure. The crude residue was purified by column chromatography on silica gel (dichloromethane/ethyl acetate, 90/10) to yield a brownish solid (4.0 g, 64.5%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.9 (t, 2H, CH_2), 3.6 (m, 2H, CH_2), 6.9-7.9 (m, 10H, aromatic H), 8.6 (t, 1H, NH), 10.8 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): 25.6 (2 × CH_2), 111.8-131.5 (aromatic CH), 135.1-136.7 (quat. aromatic C), 166.6 (C=O).

N_b-benzyltryptamine (Nbb). N_b-benzoyltryptamine (1.10 g, 4.17 mmol) was dissolved in anhydrous THF (25 mL) and cooled to 0°C. To that, $LiAlH_4$ (1.30 g, 34.5 mmol) was added cautiously. The reaction mixture was then heated to reflux for 6 hour. The reaction was then being cooled to 0°C and quenched with 10% THF/H_2O (1 mL), 10% NaOH (3 mL), and H_2O (1 mL). The light grey mixture obtained was then heated to reflux for 1.5 hour to induce the precipitation of white solid. The mixture was then filtered and the filtrate was concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (ethyl acetate/methanol, 70/30) to yield a yellowish oil (0.61 g, 67.1%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.8 (m, 4H, 2 × CH_2), 3.7 (s, 2H, CH_2), 6.9-7.4 (m, 10H, aromatic H), 7.6 (d, 1H , NH), 10.8 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): 25.8 (CH_2), 50.0 (CH_2), 53.3 (CH_2), 111.7-128.5 (aromatic CH), 136.7-141.3 (quat. aromatic C).

N_b-benzyl-1,2,3,3a,4,5-hexahydrocanthin-6-one (Nbc). To a stirred solution of N_b-benzyltryptamine (0.7 g, 2.8 mmol) in benzene; dioxane (65 mL 6:4 v/v), a-ketoglutaric acid (1.03 g, 7.0 mmol) was added. The reaction mixture was heated to reflux under $N_{2(g)}$ atmosphere using a Dean-Stark trap for water removal for 4 hour. Upon completion, the reaction mixture was cooled and the solvent was removed under reduced pressure. The residue was then dissolved in a mixture of CH_2Cl_2 and saturated aqueous $NaHCO_3$. The mixture was then being extracted with dichloromethane (3 × 30 mL), dried over anhydrous K_2CO_3, and the solvent was then removed under reduced pressure. The crude residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate, 75/25) to yield a light green solid (0.31 g, 48.9%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.8-2.1 (m, 1H, CH_2), 2.4-3.0 (m, 6H, 3 × CH_2), 3.3 (m, 1H, CH_2), 3.4 (d, 1H, CH_2Ph), 3.6 (d, 1H, CH), 4.3 (d, 1H, CH_2Ph), 7.3-8.5 (m, 8H, aromatic H); ^{13}C NMR ($CDCl_3$, 75 MHz): 21.3 (CH_2), 27.4 (CH_2), 32.9 (CH_2), 50.0 (CH_2), 57.2 (CH_2Ph), 57.7 (CH), 113.5-129.3 (aromatic CH), 134.9-138.4 (quat. aromatic C), 168.1 (C=O).

1,2,3,3a,4,5-hexahydrocanthin-6-one (Nbd). A solution of N_b-benzyl-1,2,3,3a,4,5-hexahydrocanthin-6-one (0.5 g, 1.58 mmol) dissolved in benzene (20 mL) was added to a slurry of Pd/C (10%- 1.0 g) and ammonium formate (1.3 g, 20.7 mmol) in anhydrous methanol (20 mL). The mixture was heated to reflux for 36 hour. Ammonium formate (0.5 g) was added in every 6 hour and Pd/C (0.5 g) was added in every 12 hour, into the reaction mixture. The mixture was then

filtered through Celite and the filter cake was rinsed with dichloromethane until TLC indicated complete removal of the product. Aqueous ammonia was added to the organic phase until pH 10 and being extracted with dichloromethane (3 × 30 mL), dried over anhydrous K_2CO_3, and the solvent was then removed under reduced pressure. The crude residue was purified by column chromatography on silica gel (ethyl acetate/methanol, 90/10) to yield a green oil (0.12 g, 33.6%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.6-1.8 (m, 1H, CH_2), 2.2-2.3 (m, 1H, CH_2), 2.5-3.2 (m, 6H, 3 × CH_2), 3.4-3.6 (m, 1H, CH_2), 3.8-3.9 (m, 1H, CH_2), 7.2-7.4 (aromatic H), 8.3 (d, 1H, NH); ^{13}C NMR ($CDCl_3$, 75 MHz): 21.0 (CH_2), 28.8 (CH_2), 33.1 (CH_2), 43.6 (CH_2), 50.3 (CH_2), 112.7-129.7 (aromatic CH), 134.5-135.7 (quat. aromatic C), 168.1 (C=O).

Towards canthin-6-one (Nbe/4). To a stirred solution of 1,2,3,3a,4,5-hexahydrocanthin-6-one (0.12 g, 0.53 mmol) in a solution of benzene:toluene (8 mL:3 mL), commercially available MnO_2 (0.07 g, 0.80 mmol) was added. The mixture was stirred vigorously at reflux under $N_{2(g)}$ atmosphere for 60 hour. In every 20 hour and 48 hour, MnO_2 (0.07 g) was added into the reaction mixture. The mixture was then filtered and rinsed with hot benzene. The filtrate was then concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (ethyl acetate/methanol, 80/20). ^{1}H NMR ($CDCl_3$, 300 MHz): 3.3 (t, 2H, CH_2), 3.6 (t, 2H, CH_2), 7.5-8.6 (aromatic H).

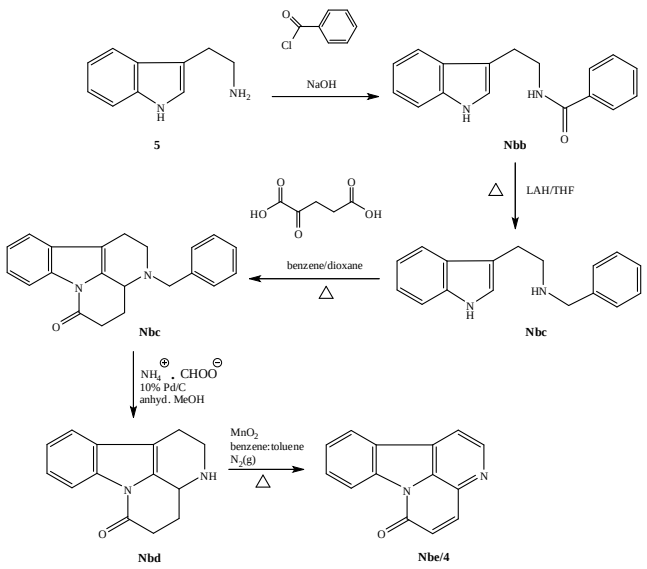
3.0 RESULTS AND DISCUSSION

The total synthesis of canthin-6-one attempt was explored in PS condensation reaction through carboxyl mediated approach according to the work of Czerwinski *et al*., (2003). In a contemplation of its viability in our organic synthesis laboratory, the biomimetic-styled reaction was carried out to enable further derivation and structural modification of β-carboline indole alkaloids to be performed. As Pictet-Spengler is a versatile reaction in organic synthesis chemistry, the study would provide information regarding canthine mechanism reaction and influential factors.

Table 1 presents yield of product at each step. The key reaction, PS provided only 48.9 % of intended product. At the end, canthin-6-one (**Nbe**) was obtained at 0.6 % only.

Table 1 Percentage of yield in the synthesis of canthin-6-one

No.	Compounds	Percentage of yield (%)
1.	Nb	64.5
2.	Nbb	67.1
3.	Nbc	48.9
4.	Nbd	33.6
5.	Nbe	0.60



Scheme 2 Total synthesis of canthin-6-one adapted from Czerwinski *et al*., (2003)

^{1}H-NMR analysis of every product at every step was performed to confirm the identity of the products as described in the report of Czerwinski *et al*., (2003). For starter, the multiple peaks at 7.0 to 7.5 ppm of 5 protons in the benzene ring observed in tryptamine (**5**) should be preserved throughout the reactions as well as the secondary amine in the pyrrole ring (10.8 ppm). As the reaction is expected to occur at the aliphatic primary amine, its peak at 2.5 ppm should move downfield due to deshielding effect. Thus, in the second reaction, **Nb** was identified through the presence of an amide bond at 8.6 ppm and an increase in the number of protons in the aromatic region from 7.0-8.0 ppm due to an addition of benzene ring. Presently, the aliphatic chain is the main link where both methylene protons are found at 2.7-2.9 ppm as multiplets. It is apparent that tryptamine had not completely reacted as the primary amine peak at 2.5 ppm is still detectable. In the reduction reaction of the amide bond of **Nbb**, a 2H singlet was observed at 3.8 ppm indicating methylene proton in the deshielded position in place of carbonyl bond. In the key reaction of PS, formation of additional rings is implied by loss of cyclic secondary amine at 10.8 ppm and aliphatic secondary amine at 2.0 ppm in addition to four pairs of methylene proton at 2.0 to 4.3 ppm in multiple splittings. Furthermore, the increase in number of protons corresponds to **Nbc**. In the next reaction, the side aromatic ring was eliminated, thus, the number of protons in the aromatic shift region has decreased to four. The breaking of linkage at cyclic amine is detected by the presence of a singlet proton at 2.0 ppm. In the last reaction, dehydrogenation of ring C and D was not successful as doublet peaks supposed to be found in the region of 6.5-8.5 ppm are not present. Therefore, **Nbe** is not canthin-6-one. The opening reaction in Czerwinski's synthesis of canthin-6-

one employs benzoyl chloride and reduction using lithium aluminium hydride (LAH) [16].

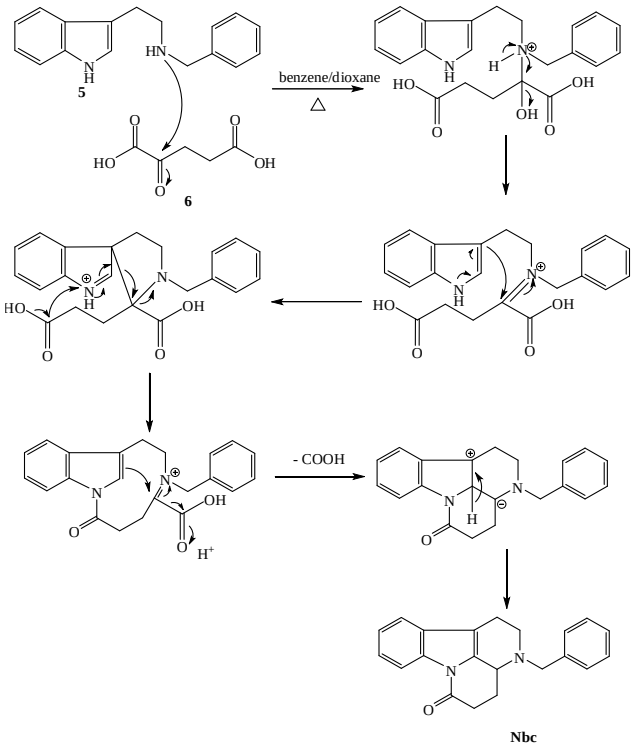
As the aim of the reaction is to protect the nitrogen atom in the aliphatic chain and formation of ring D at indole ring, the temperature for reaction of N_b-benzoyltryptamine was set at -30°C. Hence, the substituent of nitrogen atom at ethyl side chain would influence the reactivity of the intermediate iminium ion which directly set the efficiency of the overall reaction [17]. Generally, a lower pK_a value of an amine is indicative of higher electrophilicity, hence, a more reactive iminium ion. Therefore, a benzoyl (pK_a of Bn ≈ 9.5) substituent would lessen the reactivity of the iminium ion [18].

The following reaction in Czerwinski's synthesis of canthin-6-one is deoxygenation of the side chain by lithium aluminium hydride (LAH) in tetrahydrofuran (THF). $LiAlH_4$ is a reducing agent which is highly specific, reacting completely at room temperature and vigorously in the presence of water composed by Finholt in 1947 [19]. $LiAlH_4$ acts similarly as Grignard reagents. The reduction of the carbonyl bond is an addition nucleophilic reaction. The withdrawal of electrons to the oxygen atom converts the carbon atom to a carbocation. This electrophilic centre attracts the hydride of LAH eventually forming an alkoxyaluminate ion. Hence, the initial reaction should be devoid of water molecules as water may act as a nucleophilic agent and intrude which would produce unintended compounds. On the other hand, water is required to release the organic alcohol group from the metallic agent in the second step.

Cook began Pictet-Spengler study aiming at improving its stereospecificity. Since, this step is deemed the determining step; measures were taken by Cook's research group to improve its yield. In his report, Cain mentioned the successful application of benzene/dioxane, an aprotic solvent combination with 2-ketoglutaric acid (**6**). In a surprise discovery, they have found that the aprotic media worked well when the reaction was conducted at high temperature[20]. This finding has been reconfirmed when another study has indicated that temperature, amount of acid and reaction period would influence yield. In addition, products with opposite configuration of stereogenic-centre might also be formed [21].

The last reaction in Czerwinski's synthesis of canthin-6-one involves dehydrogenation in ring C and D with manganese dioxide. In Czerwinski's method, manganese dioxide was prepared in-situ whereas, in this study, commercially available manganese oxide has been used. Active manganese dioxide has been classified as a selective oxidant for alcohols transformation to aldehydes or ketones [22]. After a comprehensive kinetic study, fresh precipitated manganese dioxide is suggested for an effective reaction after finding that commercially available manganese dioxide has lower to nil activity. Active manganese dioxide can be prepared according to Attenburrow's method. Attenburrow's manganese dioxide possess high concentration of labile hydroxy radicals [23].

In Scheme 3, a PS mechanism for Czerwinski's synthesis is suggested based on Cain's report that in his reaction, 1-propionic acid (CH_3CH_2COOH) intermediate was isolated.



Scheme 3 Possible Mechanism of PS reaction in Canthin-6-one Synthesis

4.0 CONCLUSION

The key reaction in the synthesis of canthin-6-one is a Pictet-Spengler reaction employing tryptamine as the starting material and 2-ketoglutaric acid as reactant. Czerwinski's reaction was initiated by protecting the aliphatic nitrogen atom. The yield acquired is comparatively low as very low temperature is the main factor which affects the activity of the imine ion. The reaction continued by removing carbonyl group with LAH. Here, the yield obtained is low due to the presence of water since the reaction must be conducted devoid of water. The next step is the key reaction of PS condensation. Apparently, temperature, amount of acid and reaction period would influence yield of the reaction. Temperature is also the influencing factor in CTH reaction as the yield is alarming low. In the final step, canthin-6-one was not produced as commercial manganese dioxide has been used. Oxidation which utilises manganese dioxide requires the catalyst to be prepared fresh.

Pictet-Spengler reaction is found to be viable to be performed routinely in our laboratory for structural modification work of canthin-6-one. Even though the current yield is sufficient, it would be better once the conditions of the strategy have been improved.

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