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STRUCTURAL MODIFICATION OF CYTOTOXIC SERRATENEDIOL FROM LYCOPODIACEAE PLANT

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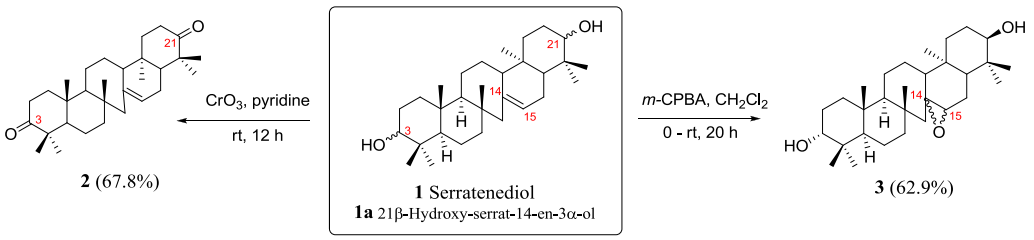
Abstract: Serratenediol (**1**)**,** cytotoxic natural product, from *L.phlegmaria* (traditional herbal medicine) were isolated and purified by chromatographic method. Structural modification of isolated serratenediols (**1**) was studied by oxidation reaction, which included the oxidation of hydroxyl groups and epoxidation of carbon double bond, to provide the new carbonyl compound (**2**) and oxirane derivatives (**3**). The obtained product **2** and **3** were identified by spectroscopic method. The carbonyl product **2** was obtained in 67.8% by using chromium trioxide in the presence of pyridine at room temperature. Meanwhile, the epoxidation reaction was carried out by using *meta-chloroperbenzoic acid* to produce product **3** in 62.9%. This work achieved to find the reaction conditions to create new derivatives of serratenediol (**2**, **3**).

Keywords: Serratenediol, Lycopodiaceae, structural modification, semi-synthesis, cytotoxicity

บทคัดย่อ: เซอราทีนไดออล (1) เป็นสารผลิตภัณฑ์ธรรมชาติที่มีความเป็นพิษต่อเซลล์มะเร็ง ถูกแยกและทำให้บริสุทธิ์โดยใช้เทคนิคโครมาโทกราฟีจากต้นช้องนางคลี่ ซึ่งเซอราทีนไดออล (1) ที่แยกได้ถูกนำมาดัดแปลงโครงสร้างด้วยปฏิกิริยาออกซิเดชัน โดยการออกซิเดชันของหมู่ไฮดรอกซิลและการทำปฏิกิริยาอีพอกซิเดชันตำแหน่งพันธะคู่ของคาร์บอน ได้ผลิตภัณฑ์เป็นสารประกอบคาร์บอนิล (2) และอนุพันธ์ออกซิเรน (3) สารประกอบคาร์บอนิลที่เตรียมได้ คิดเป็นร้อยละผลได้เท่ากับ 67.8 โดยใช้ โครเมียมไตรออกไซด์ ในสภาวะที่มีไพริดีน ที่อุณหภูมิห้อง ในขณะที่ปฏิกิริยาอีพอกซิเดชันทำได้โดยใช้ กรดเมตา-คลอโรเปอร์เบนโซอิก โดยให้ร้อยละผลได้ของผลิตภัณฑ์ 3 เท่ากับ 62.9 ในการศึกษาครั้งนี้ประสบความสำเร็จในการหาสภาวะที่ใช้ในการสังเคราะห์อนุพันธ์ใหม่ของเซอราทีนไดออล

คำสำคัญ : เซอราทีนไดออล, ไลโคโพเดียเซอิ, การดัดแปลงโครงสร้าง, การสังเคราะห์แบบกึ่ง, ความเป็นพิษต่อเซลล์มะเร็ง



INTRODUCTION

3,21-Dihydroxy-serrat-14-en or Serratenediol (**1**) is a naturally occurring triterpene, belonging to serratene-type triterpenoid. Structurally, **1** is a fused-pentacyclic triterpene with central seven membered ring C, possessing a double bond between C-14 and C- 15, seven tertiary methyl groups and two hydroxyl groups at C-3 and C-21 (with different configuration). This compound was mainly isolated from plants belonging to Pinaceae, and Lycopodiaceae families such as *L. Squarrosum*, *L. Nummuralifolium* Blume and *L. Phlegmaria.* (Boonya-udtayan *et al*., 2017; Ham *et al*., 2012; Tanaka *et al*., 2003; Wittayalai *et al*., 2012; Yan *et al*., 2008; Zhang *et al*., 2014; Zhou *et al*., 2004)

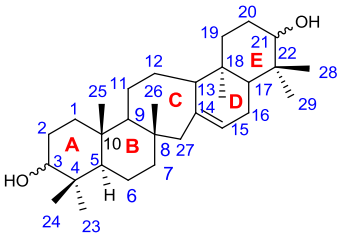


Figure 1. 3,21-Dihydroxy-serrat-14-en or Serratenediol (**1**) structure

Previous studies found that serratenediol (**1**) and its derivatives are biologically active compounds. 21β-Hydroxy-serrat-14-en-3α-ol (**1a**), lycophlegmariols B (**4**) and lycophlegmariols D (**5**) are cytotoxic compounds against MOLT-3 cells (acute lymphoblastic leukemia) with IC_{50} of 2.9, 14.7 and 3.0 µM, respectively, (Wittayalai *et al*., 2012) while lycophlegmarin (**6**) exhibited inhibitory effects against BEL 7402 cells. (Shi *et al*., 2005). Lycocavanol (**7**) was found as acetylcholineesterase inhibitor.(Yan *et al*., 2005) Lycernuic acid C (**8**) disclosed inhibitory effects against *Candida albicans* secreted aspartic protease (SAP).(Zhang *et al*., 2002) Moreover, serratenediol derivatives with oxirane ring in its structure, compound **9** and compound **10**, were found as cancer chemopreventive agents. (Doi *et al*., 2010; Tanaka *et al*., 2003; Tanaka *et al*., 2006; Tanaka *et al*., 2001)

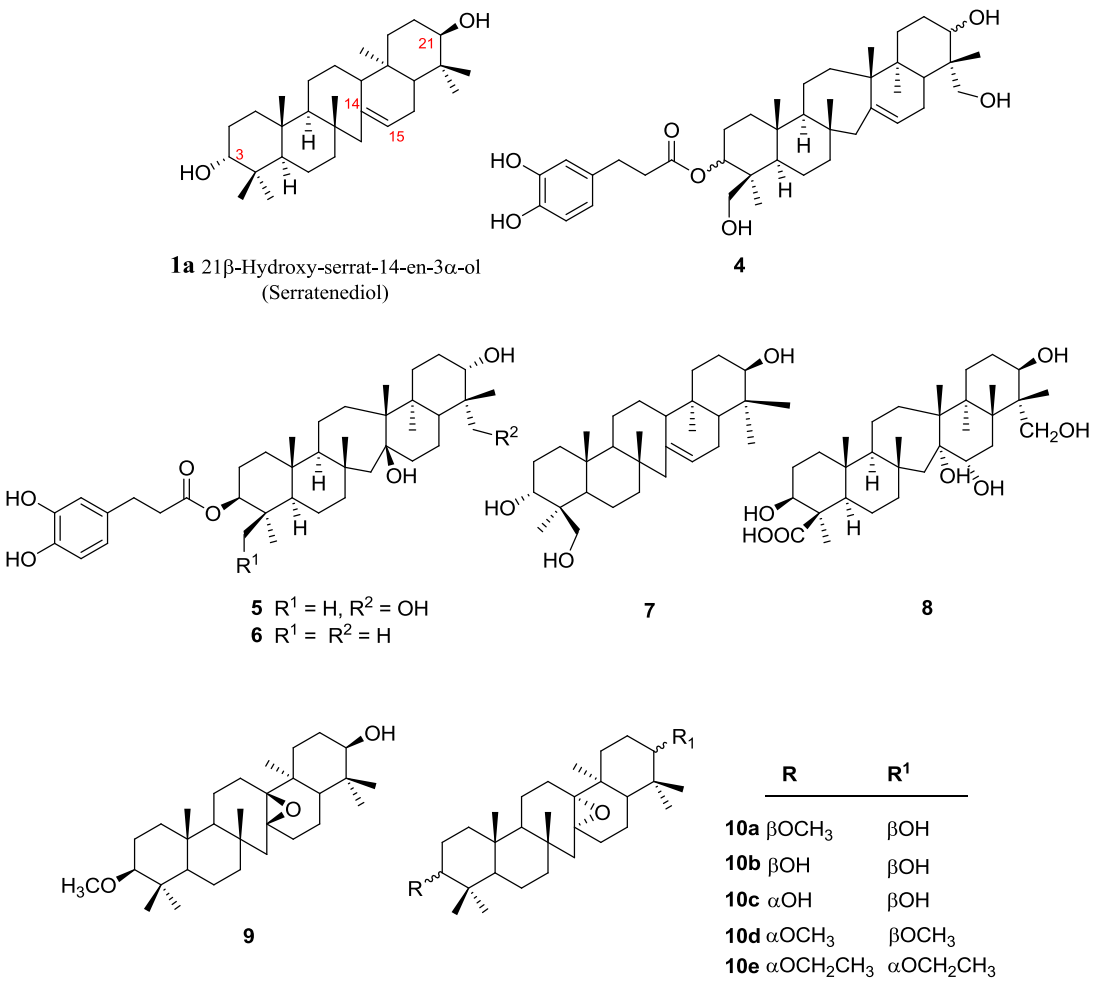


Figure 2. Biologically active Serratenediol (**1a**) and their derivatives (**2-8**)

Although the promising biological activities of serratenediol derivatives were demonstrated, the medicinal chemistry studies is limited by the available library of analogues in order to find the lead compounds including the lacking of idea of mechanism of action. In this sense, the increasing of serratenediol analogues can enhance the potential to find a new lead compound and develop more effective compounds. In this study, serratenediol (**1a**), isolated from *L.Phlegmaria* was modified by oxidation reaction, which consist of the oxidation of hydroxyl groups at C-3 and C-21 and epoxidation of double bond to create oxirane ring. (Figure 3)

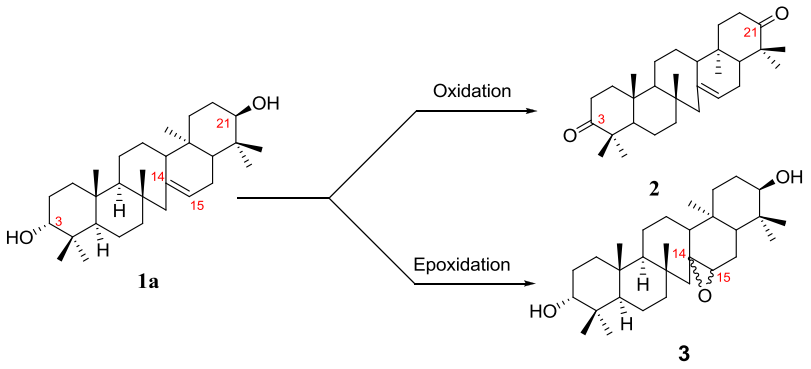


Figure 3. Structural modification of serratenediol (**1a**)

MATERIALS AND METHODS

General experimental procedure

FT-IR spectra were acquired using a Perkin Elmer (Spectrum model spectrum two). ^{1}H Nuclear magnetic resonance (^{1}H NMR) and ^{13}C Nuclear magnetic resonance (^{13}C NMR) spectra were measured in pyridine-d_5, $CDCl_3$ and CD_3OD on a Bruker AV 300 MHz and 75 MHz, respectively. High resolution mass spectra were obtained on Bruker Daltonics MicroTOF instrument using atmospheric pressure chemical ionization (APCI) in either the positive or negative ion mode. BUCHI Rotavapor R-210/215 was required for solvent residue evaporation. Silica gel (70-230 mesh, Merck) was used for column chromatography. TLC experiments were performed on silica gel 60 GF_{254} plates (TLC aluminum sheet) which were detected by heating TLC sprayed with *p*-anisaldehyde stain. All commercially required reagents are laboratory and analytical grade using without purification. All solvents were distilled prior to use.

Isolation and purification of serratenediol

Serratenediol (**1a**), starting material, was extracted from *L.Phlegmaria* by soaking in methanol (CH_3OH) at room temperature as our previous works. (Wittayalai, et al., 2012) The obtained crude extract (40.0206 g) from *L.Phlegmaria* was separated by silica gel column chromatomatography using methanol : dicloromethane (0.25 : 99.75, v/v) to provide eight fractions (F1-F8). Serratenediol (**1a**) was detected by TLC stained by *p*-anisaldehyde which was found in F4-F8. The fractions containing serratenediol (F4-F8), especially F7, were further purified by using a combination of silica gel column chromatography (CH_3OH : CH_2Cl_2, 0.25 : 99.75) and recrystallization by ethylacetate.

The obtained serratenediol (**1a**) was isolated as white solid. Melting point : >300 ^{o}C). IR (FT-IR): ν_{max} 3369, 2924, 1439, 1382 cm^{-1}. ^{1}H-NMR (300 MHz, Pyridine-d5) : δ = 5.95 (br s, 1H; OH), 5.86 (s, 1H; OH), 5.48 (br s, 1H), 3.45– 3.61 (m, 2H), 2.33 (d, *J* = 15 Hz, 1H), 2.18 (d, *J* = 21.9 Hz, 1H), 1.71 – 2.06 (m, 10H), 1.36 – 1.54 (m, 4H), 1.25 (s, 3H), 0.79 – 1.25 (m, 8H), 1.20 (s, 3H), 1.11 (s, 3H), 1.07 (s, 3H), 0.95 (s, 3H), 0.88 (s, 3H), 0.79 (s, 3H) ppm. ^{13}C-NMR (75 MHz, Pyridine-d5) : δ = 138.7 (C), 122.7 (CH), 78.2 (CH), 77.9 (CH), 62.9 (CH), 57.5 (C), 56.4 (CH_2), 56.0 (CH), 50.0 (CH), 45.5 (CH_2), 39.6 (C), 39.4 (C), 39.0 (C), 38.4 (CH_2), 37.5 (C), 37.4 (CH_2), 36.4 (C), 28.7 (CH_3), 28.5 (CH_2), 28.4 (CH_2), 28.2 (CH_3), 27.5 (CH_2), 25.5 (CH_2), 24.6 (CH_2), 20.1 (CH_3), 19.3 (CH_2), 16.3 (CH_3), 16.0 (CH_3), 15.4 (CH_3), 13.7 (CH_3). HRMS m/z calcd for $C_{30}H_{54}NO_2$ $[M+NH_4]^+$: 460.4142; found: 460.4149.

Structural modification

Oxidation of hydroxyl groups (synthesis of compound 2) (Table 1, entry 6)

The starting material **1** (103.6 mg, 0.2340 mmol) in anhydrous pyridine (1.0 mL) was treated with 0.5 M CrO_3 in pyridine solution. The reaction was stirrer under room temperature for 12 hours by detection with TLC (2% $CH_3CO_2C_2H_5$: CH_2Cl_2 mobile phase system). The reaction was quenched with water (10.0 mL) and extracted with CH_2Cl_2 three times. The combined organic layer was washed by water, brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to provide crude reaction (142.7 mg). The crude reaction was further purified by silica gel column chromatography (2% $CH_3CO_2C_2H_5$: CH_2Cl_2) to afford **2** (69.6 mg, 67.8%) and **9** (1.6 mg, 1.6%)

The oxidized product (**2**) was obtained as white solid. Melting point : 200 - 204 ^{o}C. IR (FT-IR): ν_{max} 2964, 1706, 1441, 1387 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$) : δ = 5.40 (s, 1H), 2.70 – 2.81 (dt, *J* = 5.4, 14.4 Hz, 1H), 2.44 – 2.50 (m, 2H), 2.22 – 2.30 (td, *J* = 3.6, 14.1 Hz,

2H), 2.12 – 2.20 (m, 1H), 19.5 – 2.07 (m, 4H), 1.87 (s, 1H), 1.73 – 1.87 (m, 2H), 1.54 – 1.70 (m, 3H), 1.42 – 1.51 (m, 5H), 1.17 – 1.30 (m, 3H), 1.09 (s, 3H), 1.08 (s, 3H), 1.05 (s, 3H), 1.04 (s, 3H), 0.93 (s, 3H), 0.89 (s, 3H), 0.87 (s, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$) : δ = 218.1 (C=O), 216.9 (C=O), 137.9 (C), 122.3 (CH), 62.1 (CH), 56.5 (CH), 55.8 (CH_2), 55.2 (CH), 51.2 (CH), 47.6 (C), 47.3 (C), 44.2 (CH_2), 39.5 (CH_2), 38.4 (CH_2), 37.8 (C), 37.0 (C), 36.2 (C), 34.7 (CH_2), 34.1 (CH_2), 27.7 (CH_2), 26.9 (CH_3), 25.5 (CH_2), 24.5 (CH_3), 24.5 (CH_2), 21.5 (CH_3), 21.0 (CH_3), 20.1 (CH_2), 19.3 (CH_3), 15.8 (CH_3), 12.9 (CH_3). HRMS m/z calcd for $C_{30}H_{50}NO_2$ [M+NH_4^+] : 456.3836; found : 356.3843.

Oxidation of carbon double bond (Synthesis of compound 3) (Table 2, entry 8)

A solution of starting serratenediol (**1**) (100.6 mg, 0.22 mmol) in CH_2Cl_2 (10.0 mL) was cooled to 0 ^oC and then added with *m*-chloroperbenzoic acid (*m*-CPBA) (70.1 mg, 0.44 mmol). The reaction was stirred under room temperature for 20 hours which the reaction was followed by TLC (15% $CH_3CO_2C_2H_5$: CH_2Cl_2). After 20 hours, the reaction was quenched by water then extracted with CH_2Cl_2 three times. The organic layers were combined and washed with water, brine, respectively. The organic part was dried over anhydrous Na_2SO_4, filtered, and removed solvent under reduced pressure to provide crude reaction. The crude reaction was purified by silica gel column chromatography (15% $CH_3CO_2C_2H_5$: CH_2Cl_2) to provide desired product **3** as a white solid (57.7 mg, 62.9%) with starting material **1a** (45.6 mg, 28.6%).

Compound **3** was obtained as white solid. Melting point = 254 ^oC. ^{1}H NMR (δ, ppm) ($CDCl_3$, 300 MHz): δ = 3.17 – 3.22 (dd, *J* = 11.4, 6.6 Hz, 2H), 2.84 (br s, 1H), 1.09 (s, 3H), 0.97 (s, 6H), 0.85 (s, 3H), 0.83 (s, 3H), 0.77 (s, 3H), 0.73 (s, 3H), 0.70 – 2.08 (m, 26H). HRMS m/z calcd for $C_{30}H_{54}NO_3$ [M+NH_4^+] : 476.4098; found: 476.4088.

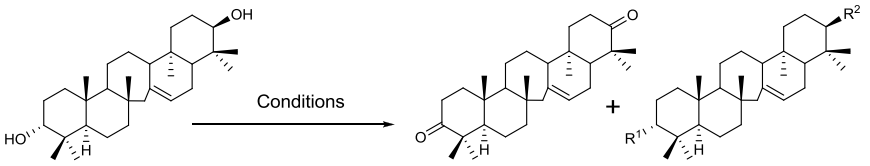
RESULTS AND DISCUSSION

Crude extract of *L.Phlegmaria* (40.0206 g) was fractioned through a silica gel column using methanol : dichloromethane (0.25 : 99.75, v/v) as the eluents in order to isolate serratenediol starting material (**1a**) which provide eight primary fractions (F1-F8). Serratenediol was detected by TLC, stained with *p*-anisaldehyde, and found in F4-F8 which major part of serratenediol was appeared in F7. The fractions containing serratenediol were further purified by silica gel chromatography (CH_3OH : CH_2Cl_2, 0.25 : 99.75) and recrystallization with ethylacetate. The major part of purified serratendiol was obtained from F7 as white solid (893.1 mg).

Structural modification of isolated serratenediol was carried out using oxidation reaction involving the oxidation of hydroxyl groups and epoxidation. The oxidation of hydroxyl groups were tried in order to create carbonyl derivative. At the beginning, no any desired product was obtained when oxone or H_2O_2 were employed as the oxidizing agents. (Table 1, entry 1-3) The oxidation of the hydroxyl groups of **1** was achieved when CrO_3 in pyridine was utilized under room temperature, affording compound **2** and **9**. (Table 1, entry 4) The product **2** was inferred by NMR data. Two signals of carbonyl carbons (δ = 218.1 and δ= 216.9) were observed instead of oxymethines carbon (δ = 138.7 and δ = 122.7) appearing in serratene starting material. This result corresponded to ^{1}H NMR, in which the signal of two protons connecting to oxygenated carbon were disappeared (δ = 3.45 – 3.61). (*See materials and methods*) Not only compound **2** was obtained but some mono-oxidized product (**9**) also. Only one carbonyl signal was observed in NMR and IR spectrum showed both signal of carbonyl (1705.6 cm^{-1}) and hydroxyl functional group (3491.9 cm^{-1}).

The increasing of CrO_3 concentration (0.5 M) results in the higher yield (table 1, entry 6). However, the result from the condition using CrO_3 in H_2O was not satisfied which provided product in very low yield and starting material could not be recovered (table 1, entry 5).

Table 1. Structural modification of serratenediol (**1a**) by oxidation of hydroxyl groups



1a **2** **9a** R^1 = O, R^2 = OH
9b R^1 = OH, R^2 = O

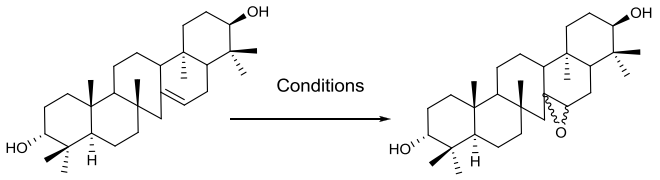
Entry	Condition	Product[a] (%yield)
1	**1a** (0.113 mmol), Al_2O_3 (1.628 mmol), Oxone (0.296 mmol), CH_3OH : CH_2Cl_2 (1 : 4) (10.0 mL), rt, 24 h.	–[b]
2	**1a** (0.113 mmol), Al_2O_3 (1.628 mmol), Oxone (0.296 mmol), CH_3OH : CH_2Cl_2 (1 : 4) (10.0 mL), reflux, 6 h.	–[b]
3	**1a** (0.113 mmol) Al_2O_3 (0.0518 mmol), H_2O_2 (0.3387), CH_2Cl_2 (10 mL), rt, 24 h.	–[c]
4	**1a** (0.113mmol), 0.2 M CrO_3 in pyridine (2.5 mL), pyridine (1.0 mL), rt, 12h.	**2** (45.4%) **9** (9.7%)
5	**1a** (0.1160 mmol) 0.2 M CrO_3 in H_2O (2.5 mL), pyridine (1.0 mL), rt, 12h.	**2** (2.2%) **9** (2.6%)
6	**1a** (0.2340 mmol) 0.5 M CrO_3 in pyridine (2.5 mL), pyridine (1.0 mL), rt, 12h	**2** (67.8%) **9** (1.6%)

[a] Structural identification by ^{1}H NMR (300 MHz, $CDCl_3$), [b]Starting material recovery by TLC identification.,
[c] Several compounds were observed from TLC without desired product.

Another modification, we paid attention to the oxidation of C-14 and C-15 double bond. The first reaction was tried by H_2O_2 in the presence of formic acid under room temperature using mixture of dichloromethane and methanol as solvent. The result had not got any change despite time increasing to four hours. (Table 2, entry 1-3) The reason involved the stability of H_2O_2 under room temperature that lead to the next attempt which was studied under low temperature (0 ^oC). This condition provided the promising result which showed no starting serratendiol left during three hours. (Table 2, entry 4) The observed product was identified as compound **3** by ^{1}H NMR which the signal of carbon double bond at δ = 5.48 ppm was replaced by broad singlet signal (br, s) of proton connecting to oxygenated carbon (C-15) at δ = 2.84 ppm. The structure of compound **3** was confirmed by HRMS which molecular weight and molecular formula was assigned as 476.4088 for $C_{30}H_{54}NO_3$; $[M+NH_4^+]$.

The reaction was tried under basic condition (NaOH) instead of acid (HCOOH) which no any desired product (**3**) was obtained (table 2, entry 5-6). Although condition using H_2O_2 as an oxidizing agent provided desired product, it required large amount (≈ 30 eq.) that cause a problem about solubility of starting material in 35% H_2O_2 solution. We next turned our attention to use meta-chloroperbenzoic acid (*m*-CPBA) as an oxidizing agent which provided the desired result.-The required product (**3**) was obtained in moderate yield (50.9, 62.9%) and starting material was also recovered which product **3** was slightly higher when increasing *m*-CPBA. (Table 2, entry 7, 8) The comparison of these two oxidized conditions found that the condition using *m*-CPBA provided a better yield and also recovered starting compound.

Table 2. Structural modification of serratenediol (**1**) by epoxidation reaction



1 **3**

Entry	Conditions	Product (%yield)[a]
1	**1a** (0.11 mmol), 35% H_2O_2 (0.10 mL), 88% HCOOH (0.10 mL), CH_2Cl_2 (5.0 mL), CH_3OH (5 drops), rt, 1.5 h	**1a** (77.0)[b]
2	**1a** (0.11 mmol), 35% H_2O_2 (0.10 mL), 88% HCOOH (0.10 mL), CH_2Cl_2 (5.0 mL), CH_3OH (5 drops), rt, 4 h	**1a** (64.8)[b]
3	**1a** (0.11 mmol), 35% H_2O_2 (0.10 mL), 88% HCOOH (0.10 mL), CH_3OH (5.0 mL), rt, 2 h	–[c]
4	**1a** (0.11 mmol), 35% H_2O_2 (0.1 mL), 88% HCOOH (0.10 mL), CH_2Cl_2 (5.0 mL), CH_3OH (5 drops), 0 ^oC, 3 h	**3** (48.8)
5	**1a** (0.11 mmol), 35% H_2O_2 (0.1 mL), 10% NaOH (0.10 mL), CH_2Cl_2 (5.0 mL), CH_3OH (5 drops), 0 ^oC, 5 h	**1a** (86.6)
6	**1a** (0.11 mmol), 35% H_2O_2 (0.1 mL), 10% NaOH (0.10 mL), CH_2Cl_2 (5.0 mL), CH_3OH (5 drops), 0 ^oC, 24 h	**1a** (77)
7	**1a** (0.2 mmol), *m*-CPBA (0.2 mmol) CH_2Cl_2 (5.0 mL), 0 $^\circ$C – rt, 20 h	**1a** (30.6) **3** (50.9)
8	**1a** (0.2 mmol), *m*-CPBA (0.4 mmol), CH_2Cl_2 (10.0 mL), 0 $^\circ$C – rt, 20 h	**1**a (28.6 **3** (62.9)

[a] Structural identification by ^{1}H NMR (300 MHz, $CDCl_3$), [b] TLC identification

[c] Several compounds were observed from TLC without desired product.

CONCLUSION

This study provided the preliminary conditions to render the new derivatives of serratendiol. New carbonyl compound (**2**) and oxirane derivative (**3**) were obtained in moderate yield by using chromium trioxide (CrO_3) and the using of *m*-CPBA, respectively. Although the products have not evaluated their biological activity yet, they were found that the products had a better solubility in organic solvent compared to the substrate. This makes it easy to study or manipulate in the next step for medicinal chemistry studies. However, the further structural modification, intensive spectroscopy, and biological study of modified compounds are an ongoing process.

ACKNOWLEDGMENTS

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