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Efficient, column-chromatography-free synthesis of Dipterocarpol succinate oxime ester salts

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Abstract

Natural-product-based drugs commonly were converted to organic or inorganic salts due to better stability, solubility or membrane-permeability of new salts compared to the drug itself. In this report, succinate oxime ester of dipterocarpol was synthesized successfully in a simple column chromatography-free isolation process. The oxime ester acid was then reacted with various organic, inorganic base to create different counterion salts. Two derivatives were shown to be stable for a period of time for future antimicrobial evaluation.

Keywords: Dipterocarpol; natural product semi-synthesis; succinate salt; antimicrobial.

Tóm tắt

Dẫn xuất muối của các hợp chất tự nhiên thường được sử dụng để tăng khả năng hòa tan trong máu và hấp phụ qua màng tế bào. Trong báo cáo này, nhóm nghiên cứu đã tổng hợp thành công dẫn xuất oxim este succinat của dipterocarpol trong điều kiện hóa học hiện đại, trong đó tối giản hóa quy trình cô lập bằng phương pháp không sử dụng cột sắc ký. Sau đó các muối của dipterocarpol oxim succinat ester acid tiếp tục được điều chế. Kết quả nghiên cứu thu được hai muối bền cho các nghiên cứu sâu về hoạt tính sinh học sau này.

Từ khóa: Dipterocarpol; bán tổng hợp hợp chất tự nhiên; muối succinat; hoạt tính sinh học.

1. Introduction

Plant extracts used in traditional Chinese medicine have long been the main sources of structurally complex molecules, biological activity, drug, and synthetic starting material. As a consequence, molecules bearing dammarane-core are widely available in large quantities from various parts of different plant

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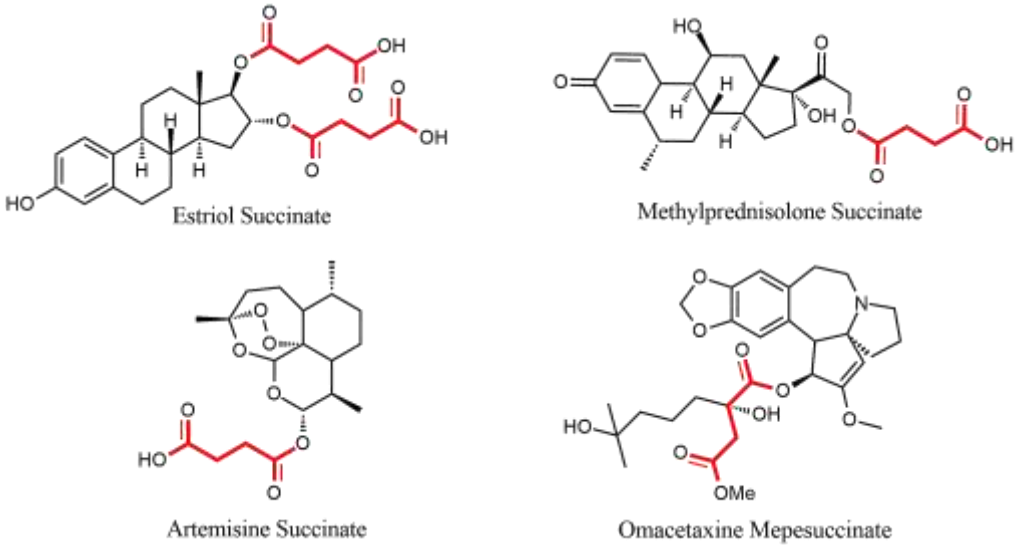
species such as bark, leaf, root, and resin [1-4]. Especially *Dipterocarpus alatus resin*, in which dipterocarpol, a dammarane triterpenoid, can be isolated up to 30% yield. Previous study from various research groups revealed significant biological activity of dipterocarpol derivatives such as antimicrobial, anti-inflammation, and anti-cancer [5, 6]. Therefore, the development of more dipterocarpol hybrid is crucial in pharmaceutical, agrochemical industry.

However, there is no to rare report about the hybrid of dipterocarpol with succinic acid despite the fact that succinic acid, along with its esters and salts, plays an crucial roles in body metabolism such as making ATP, regulation of cellular function [7]. Furthermore, there is a myriad of regulated drug - succinate hybrid has been developed and reported which indicated the predominant of succinate derivatives in the pharmaceutical industry (**Scheme 1**). Additionally, oxime esters also exhibited promising biological activities during the pharmaceutical discovery [8-14].



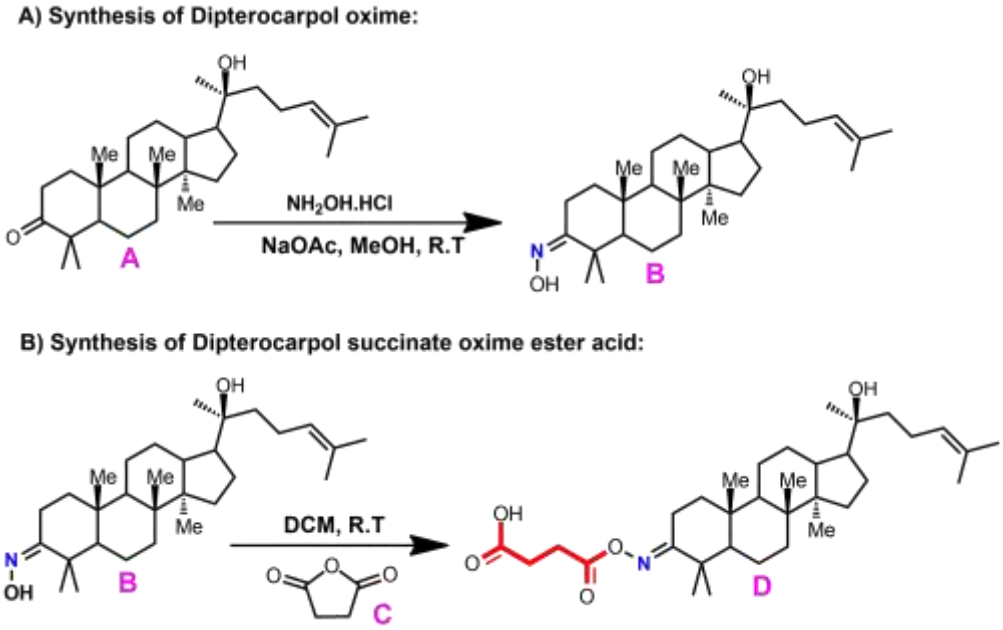
Scheme 1. Pharmaceutical (Natural Products) - Succinate Hybrid.

2. Our approach

Given the ubiquity of pharmaceutical and life-science chemical that exists as inorganic, organic salt instead of free carboxylic acid due to their roles in human body such as nervous impulses transmission, lower toxicity, higher blood solubility [15, 16], we propose further transformations of dipterocarpol succinate oxime ester (free acid) to corresponding salts will benefit further biological evaluation. However, previous reports in the synthesis of dipterocarpol derivatives required copious, stoichiometric amount of reagent, solvent, toxic reagent and harsh condition such as ozone, hydrazine, strong acid, low reaction temperature (-78 ^{o}C). Herein, we would like to report a green, high yield, column-chromatography-free synthesis of dipterocarpol succinate oxime ester and its corresponding salt. We believe this research will circumvent those afore mentioned problems and develop new, efficient antimicrobial candidates by: (i) performing the reaction at room-temperature; (ii) eliminating the use of coupling reagent or pre-functionalized reagent; (iii) reducing the use of solvent during purification step by developing chromatography-free reaction.

3. Results and discussion

Our synthetic process started with the isolation of dipterocarpol, a highly abundant material in Dipterocarpaceae resin. This commercial resin was first washed several times with hexane to remove oil, followed by recrystallization in hot ethanol to obtain the crude dipterocarpol. After further recrystallization in the same solvent, pure dipterocarpol could be afforded with considerable amount. The compound was confirmed by ^{1}H-NMR and ^{13}C-NMR spectroscopy which matched previous reports and commercial source. The isolated dipterocarpol was then mixed with hydroxylamine hydrochloride, sodium acetate, and methanol, and heated at 40^{o}C for 3h to generate the oxime derivative. The reaction proceeded smoothly and gave 87% yield oxime **B** after simple recrystallization (**Scheme 2A**)

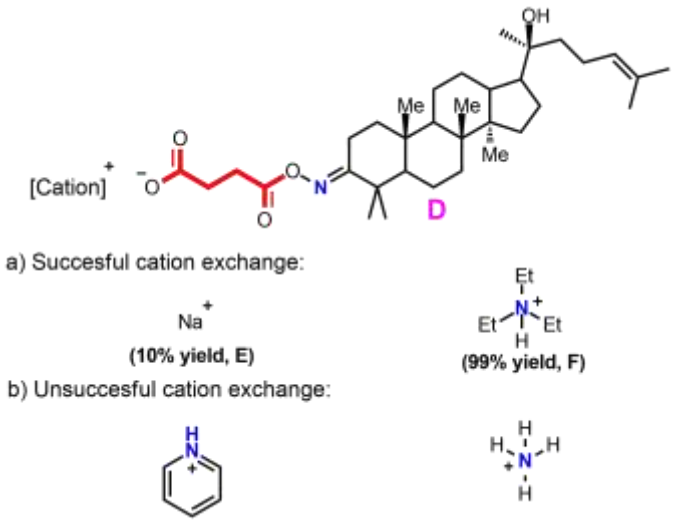


Scheme 2: Preparation of dipterocarpol oxime and dipterocarpol succinate oxime ester acid

Subsequently, the precursor succinate oxime ester acid **D** was synthesized by mixing dipterocarpol oxime **B** with succinic anhydride and pyridine in dichloromethane at room temperature. The reaction, however, did not come to completion and gave low yield in addition to a complicated work up process. Furthermore, we realized that even with the presence of pyridine, the product was in the form of acid but not pyridine salt. This led us to try new procedure without using pyridine. Interestingly, when we run the reaction in the absence of pyridine, the acid D could be still obtained in 58% yield. Any oxime **B** residue was simply removed by washing crude product several times with hexanes and avoided a long work up and column chromatography isolation process. (**Scheme 2B**).

Encouraged by these successful primary experiments, we began to convert the free acid dipterocarpol succinate oxime ester **D** into corresponding salts. We chose ammonium, triethylammonium, pyridinium, sodium, and potassium as the counterion of acid **D** due to their widely appearance in pharmaceutical or physical properties (**Scheme 3**) [17]. We conduct the reaction using ammonia solution but the starting material **D** decomposed. Further control experiment revealed that **D** was mildly sensitive to moisture. Therefore, we performed

the reaction using ammonia soluble in ethyl acetate, but the reaction did not proceed even after we change the reaction conditions such as solvent (DCM) and additive (using anhydrous Na_2SO_4).



Scheme 3: Ion exchange reaction affording counterion dipterocarpol succinate oxime ester salts

Nevertheless, when sodium hydroxide (NaOH) was used in Et_2O, we could generate the formation of the sodium salt derivative, even though with low yield (10% yield). Notably, the major by-product we observed was dipterocarpol oxime **B** which resulted from the hydrolysis of labile oxime ester **D** and therefore, limited the emergence of the desired product. On the other hand, this hydrolysis was surprisingly not occurred when we applied triethyl amine in the reaction conditions. As a result, the triethylammonium salt of acid **D** was afforded in almost quantitative yield. The expected salts precipitated out of triethylamine solvent and was comfortably collected by filtration. Our efforts to produce other salts such as potassium and pyridinium were unsuccessful.

4. Conclusion

In conclusion, this report focused on the efficient synthesis and preparation of dipterocarpol succinate oxime ester acid derivative and its salts under mild, green, and column-chromatography-free isolation. We believe this method will be suitable for large-scale synthesis, thus benefiting the pharmaceutical industry in search for biologically active compounds. Further biological evaluation of these potential compounds is in progress.

Experimental Section

Isolation of Dipterocarpol (A) from Dipterocarpaceae (Dau Rai) resin: From Dipterocarpaceae pitch, the mixture was left to settle for 2-3 days, and the liquid was removed from the residue. After the addition of hexanes, two fractions were obtained: soluble fraction and insoluble fraction. The insoluble fraction was added to ethanol and heated at 60 ^oC for 30 minutes, then filtered off any insoluble residue over Buchner under reduced pressure to obtain a hot yellow solution. After that, crystallized crude dipterocarpol product was collected by filtration after cooling the solution in refrigerator. After recrystallizing the crude product a few more times in ethanol, dipterocarpol was obtained as a white needle crystal in 15% yield. The structure of

dipterocarpol was confirmed by ^{1}H and ^{13}C NMR spectroscopy. **^{1}H NMR** (500 MHz, CDCl3) δ 5.12 (ddt, J = 7.1, 5.7, 1.4 Hz, 1H), 2.50 (ddd, J = 15.7, 9.6, 7.6 Hz, 1H), 2.42 (ddd, J = 15.6, 7.7, 4.4 Hz, 1H), 2.09 – 2.02 (m, 2H), 1.92 (ddd, J = 13.2, 7.6, 4.5 Hz, 1H), 1.87 – 1.82 (m, 1H), 1.75 (td, J = 7.9, 7.2, 3.3 Hz, 2H), 1.69 (s, 3H), 1.63 (s, 3H), 1.59 – 1.55 (m, 4H), 1.51 – 1.41 (m, 7H), 1.40 – 1.21 (m, 5H), 1.15 (s, 3H), 1.08 (s, 3H), 1.04 (s, 3H), 1.00 (s, 3H), 0.94 (s, 3H), 0.89 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 218.24, 131.70, 124.68, 75.39, 55.33, 50.27, 49.99, 49.79, 47.45, 42.37, 40.45, 40.26, 39.89, 36.83, 34.53, 34.14, 31.17, 27.53, 26.70, 25.79, 25.49, 24.81, 22.57, 22.03, 21.03, 19.65, 17.75, 16.35, 16.06, 15.21.

Procedure for the synthesis of Oxime: In a 50 mL beaker, 4.4 g dipterocarpol (9.7 mmol) was taken with 40 mL methanol. This solution was heated on a magnetic stirrer at 40^{O}C to make sure all the crystal was dissolved completely. To another 100 ml beaker, 2.1g hydroxylamine hydrochloride (0.03 mol, 3 eq) and anhydrous sodium acetate (CH_3COONa) were added. This mixture was added methanol while being stirred at room temperature until it dissolved apart. After that, the solution in 50ml beaker was poured to this 100ml beaker and the mixture was stirred at room temperature until the completion of reaction. Thin layer chromatography was used to monitor the completion of the reaction. After completion of the reaction, the mixture was let in the refrigerator at 0-10^{O}C for the crystallization overnight. The day after, the mixture was filtered by buchner funnel with reduced pressure and the residue was washed with water and air dried to obtain a white solid in 87% yield (3.86 g). The Structure of dipterocarpol oxime was confirmed by ^{1}H and ^{13}C NMR spectroscopy. **^{1}H NMR** (500 MHz, CDCl) δ 5.11 (tt, J = 7.1, 1.4 Hz, 1H), 2.96 (ddd, J = 15.4, 5.9, 3.9 Hz, 1H), 2.32 – 2.22 (m, 1H), 2.04 (p, J = 7.1 Hz, 2H), 1.85 – 1.75 (m, 2H), 1.75 – 1.70 (m, 2H), 1.68 (s, 3H), 1.65 (d, J = 11.4 Hz, 0H), 1.62 (s, 3H), 1.56 – 1.42 (m, 8H), 1.38 – 1.19 (m, 6H), 1.14 (s, 6H), 1.12 – 1.05 (m, 2H), 1.05 (s, 3H), 0.98 (s, 3H), 0.94 (s, 3H), 0.86 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 167.21, 131.68, 124.69, 75.46, 56.04, 50.89, 50.29, 50.25, 49.77, 42.31, 40.48, 40.46, 40.38, 39.10, 37.18, 34.82, 31.14, 27.53, 27.29, 25.79, 25.42, 24.80, 22.86, 22.56, 21.80, 19.03, 17.75, 17.13, 16.34, 15.92, 15.40.

Procedure for the synthesis of oxime ester acid derivative (D): In a 2-dram vial equipped with a stirbar was added 45 mg of Oxime (**B**) (0.1 mmol) and Succinic acid (14.2 mg, 0.12 mmol), followed by 1.0 ml of dichloromethane. The mixture was stirred until all the reaction was dissolved. The reaction was monitored using TLC until all of dipterocarpol was consumed, after that the crude acid product was obtained. The collected solid was washed with hexanes until no oximes was detected by TLC. After removing hexanes by rotovatory evaporation under reduced pressure, the acid derivative D was isolated in 58% yield as a white solid (32.4 mg). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 12.26 (s, 1H), 5.08 (tt, *J* = 7.1, 3.6 Hz, 1H), 3.87 (s, 1H), 2.76 (ddd, *J* = 15.0, 6.3, 4.6 Hz, 1H), 2.63 (dd, *J* = 7.7, 5.2 Hz, 2H), 2.56 – 2.51 (m, 2H), 1.95 (hept, *J* = 6.1, 4.8 Hz, 2H), 1.83 – 1.67 (m, 2H), 1.65 – 1.59 (m, 5H), 1.56 (s, 3H), 1.54 – 1.45 (m, 4H), 1.44 – 1.27 (m, 6H), 1.27 – 1.19 (m, 3H), 1.15 (s, 6H), 1.07 (s, 3H), 1.01 (s, 3H), 0.99 – 0.96 (m, 1H), 0.94 (s, 3H), 0.89 (s, 3H), 0.83 (s, 3H). **^{13}C NMR** (101 MHz, DMSO) δ 175.36, 174.13, 173.88, 170.90, 130.50, 125.69, 73.43, 55.47, 50.34, 50.06, 49.25, 42.05, 41.53, 41.40, 37.04, 34.79, 31.32, 29.29, 28.99, 28.23, 27.73, 27.67, 25.99, 25.74, 24.80, 22.81, 22.71, 21.99, 19.52, 18.96, 17.98, 16.71, 16.32, 15.48.

Procedure for synthesis of dipterocarpol succinate oxime ester sodium salt (E): In a 2-dram vial was added 20 mg sodium hydroxide (0.5 mmol, 5 equiv.), 71 mg anhydrous Na_2SO_4 (0.5 mmol, 5 equiv.), followed by dipterocarpol succinate oxime ester acid (**D**) (56 mg, 0.1 mmol) and 1.0 ml of diethyl ether. The mixture was stirred for 10 minutes and monitored by TLC, the vial was then rotavaped to afford crude product. Further washing with copious amount of hexanse to wash excess decomposed oxime ester was done to afford pure product as white amorphous powder (10% yield, 5.8 mg, 0.01 mmol). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 5.14 – 4.99 (m, 1H), 3.89 (s, 1H), 2.81 (ddt, *J* = 20.9, 15.1, 4.8 Hz, 1H), 2.50 – 2.43 (m, 2H), 2.44 – 2.37 (m, 0H), 2.15 (t, *J* = 7.0 Hz, 2H), 1.94 (q, *J* = 6.0 Hz, 2H), 1.83 – 1.67 (m, 2H), 1.66 – 1.58 (m, 5H), 1.56 (s, 3H), 1.54 – 1.45 (m, 3H), 1.45 – 1.27 (m, 5H), 1.27 – 1.19 (m, 3H), 1.14 (s, 4H), 1.08 (s, 2H), 1.06 (s, 3H), 1.01 (s, 3H), 0.97 (s, 2H), 0.94 (s, 3H), 0.88 (s, 3H), 0.87 – 0.84 (m, 1H), 0.82 (s, 3H). **^{13}C NMR** (101 MHz, DMSO) δ 175.11, 174.73, 171.86, 130.49, 125.69, 73.43, 55.56, 50.34, 50.07, 49.22, 42.04, 41.55, 41.32, 37.03, 34.81, 34.66, 32.85, 31.45, 31.32, 30.48, 27.72, 27.67, 25.99, 25.72, 25.25, 24.80, 22.88, 22.71, 22.56, 21.97, 19.45, 18.95, 17.98, 16.70, 16.28, 15.49, 14.47.

Procedure for the synthesis of dipterocarpol succinate oxime ester triethylammonium salt (F): In a 2-dram vial was added 56 mg dipterocarpol succinate oxime ester (D) (0.1 mmol) and 20 ul triethylamine (0.15 mmol, 1.5 equiv.) under neat condtion . The mixture was stirred for 10 minutes and monitored by TLC until white percipitaed appeared. Further washing with copious amount of hexanes was done to afford pure product dipterocarpol succinate oxime ester triethylammonium salt as pale yellow, slightly odor crystal (99% yield, 65 mg, 0.1 mmol). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 5.13 – 4.99 (m, 1H), 2.76 (ddd, *J* = 15.0, 6.2, 4.6 Hz, 1H), 2.64 (p, *J* = 7.2 Hz, 6H), 2.50 – 2.39 (m, 4H), 1.94 (q, *J* = 6.3 Hz, 2H), 1.83 – 1.66 (m, 2H), 1.63 (d, *J* = 1.4 Hz, 3H), 1.61 (s, 2H), 1.56 (d, *J* = 1.3 Hz, 3H), 1.50 (t, *J* = 7.2 Hz, 4H), 1.44 – 1.27 (m, 6H), 1.24 (q, *J* = 3.7 Hz, 3H), 1.15 (s, 5H), 1.07 (s, 3H), 1.05 – 0.96 (m, 14H), 0.94 (s, 3H), 0.88 (s, 3H), 0.82 (s, 3H). **^{13}C NMR** (101 MHz, DMSO) δ 175.36, 173.99, 170.94, 130.51, 125.69, 73.43, 55.46, 50.34, 50.05, 49.24, 46.07, 42.04, 41.53, 41.40, 37.04, 34.78, 32.25, 31.31, 29.18, 28.34, 27.73, 27.66, 25.99, 25.75, 24.80, 22.81, 22.71, 21.98, 19.52, 18.94, 17.98, 16.71, 16.32, 15.48, 11.37.

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