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HIGHLY EFFICIENT PREPARATION OF ETHYL PIPERATE FROM PIPERINE ISOLATED FROM *PIPER NIGRUM*

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INTRODUCTION

Piper nigrum L. is a medicinal plant in the family Piperaceae. This plant is native to south India and widespread in tropical region[1)]. In Thailand, black pepper is commonly consumed as a condiment and also used as an ingredient in traditional Thai medical preparations[2)]. Piperine is the major alkaloid responsible for the pungency of black pepper. The amount of piperine varies from 5-7% in white and black pepper. Piperine has been reported to show lipid-lowering effect in drug-induced hypercholesterolemic rats[3)]. However, piperine was cytotoxic to cultured embryonic rat brain neurons and caused extensive immunotoxicological effects in mice[4, 5)]. Ethyl piperate is modified from piperine to get rid of the toxicity of the parent compound and retain its lipid-lowering effect[6)]. We are interested in studying the preparation of ethyl piperate by using synthetic method for further investigation of its other biological activities.

MATERIALS AND METHODS

Instrument and reagents Dicyclohexylcarbodiimide (DCC) and 4-dimethyl-aminopyridine (DMAP) were purchased from Aldrich (New Jersey, USA). *p*-Toluene sulfonic acid was obtained from Aldrich (New Jersey, USA). All other reagents and solvents were reagent grade and used without further purification. TLC was performed on silica gel GF_{254} plates (Merck). For column chromatography, silica gel (Merck 230-400 mesh) was used. NMR spectra were recorded with a Bruker Avance (^{1}H, 300 MHz) spectrometer. Chemical shifts are reported in ppm, and coupling constants are reported in Hz. All NMR spectra were obtained in deuterated chloroform ($CDCl_3$), DMSO-d_6 and referenced to the residual solvent peak. Mass spectra were obtained on an Agilent GC/MS 5975C.

Plant material The dried fruits of *Piper nigrum* were bought from local drugstore in Nonthaburi province, Thailand. They were identified by comparison with the specimens at the Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok. The voucher specimen of *Piper nigrum* (SRU 024) was deposited at the Faculty of Oriental Medicine, Rangsit University, Pathumthani, Thailand.

Preparation of crude extracts The dried, powdered fruits of *Piper nigrum* (100 g) were extracted with 95% ethanol (400 mL) at room temperature for 7 days. The extract was filtered with Whatman No.1 filter paper and then evaporated under reduced pressure with rotary evaporator to obtain 9 g of the final crude dark brown extract.

Isolation of piperine The crude extract (9 g) was dissolved in ethanol (30 ml). Potassium hydroxide (8.0 g) was added to the mixture and stirred until completely dissolved. Then, the mixture was filtered through Whatman No. 1 filter paper and left overnight to freely evaporate at room temperature. After this process, piperine precipitated in the mixture as brown solid compound. The crude product was washed with hot water three times to remove the water-soluble residues. The solid was recrystallized with isopropanol and kept at 15ºC for 3 days. After that, the crystals were filtered upon Whatman No. 1 filter paper to obtain yellow solid (2.9591 g) with melting point of 129-130 ºC. UV[(7)] λ_{max} 343 nm; IR[(8)] (KBr disc): 3010, 2942, 1633, 1581, 1433 cm^{-1}; ^{1}H NMR[(9)] (300 MHz, $CDCl_3$) δ [ppm]: 1.59 (m, 4H), 1.64 (m, 2H), 3.55 (d, 4H), 5.95 (s, 2H), 6.44 (d, J = 14.5 Hz, 1H), 6.74 (m, 3H), 6.89 (d, J = 8 Hz, 1H), 6.97 (s, 1H), 7.40 (qd, J = 15, 2 Hz, 1H). ^{13}C NMR[(10)] (75 MHz, $CDCl_3$) δ [ppm]: 165.3 (C-1), 148.1 (C-3'), 148.0 (C-4'), 142.4 (C-3), 138.2 (C-5), 131.0 (C-1'), 125.3 (C-4), 122.4 (C-6'), 120.0 (C-6'), 108.4 (C-5'), 105.6 (C-2'), 101.2 (C-8), 46.8 (C-9), 43.2 (C-9), 26.6 (C-11), 25.6 (C-11), 24.62 (C-10) and MS[(2)] (GC/MS): M^+ = 285.

Preparation of piperic acid To a round bottom flask (50 mL) containing 0.50 g of piperine, 20% alcoholic potassium hydroxide (30 mL) was added. The mixture was refluxed at 70 ºC for 12 h and then cooled down to room temperature. The solution was acidified with 1M HCl to pH 3.0 and then transferred to a separatory funnel. Dichloromethane (30 mL) was added to the separator to extract the aqueous layer. The extraction was repeated two times and the dichloromethane layers were collected, evaporated to obtain crude piperic acid. The crude compound was recrystallized with methanol-water

(8:2) to give crystals of piperic acid (0.2814 g, 57.61% yield) with melting point of 213-215 ºC. UV λ_{max} 340 nm; IR[11] (KBr disc): 3300-2500 (broad), 1671, 1594 cm^{-1}; ^{1}H NMR (300 MHz, DMSO- d_6) δ [ppm]: 5.92 (d, *J* = 15.1 Hz, 1H), 6.05 (s, 2H), 6.92-7.00 (m, 4H), 7.23 (s, 1H), 7.25-7.32 (m, 1H). ^{13}C NMR (75 MHz, DMSO- d_6): 167.8, 148.2, 148.0, 144.8, 139.9, 130.6, 124.9, 123.2, 121.2, 108.6, 105.8, 101.4 and MS (GC/MS): M^+ = 218.

Preparation of ethyl piperate by acid catalyzed esterification To a round bottom flask (50 mL) containing piperic acid (1.0003 g) in toluene (10 mL), ethanol (1.0 mL) and *p*-Toluene sulfonic acid (0.05 g) were added. The flask was connected with Dean-Stark Apparatus to remove the water from the reaction. The reaction mixture was refluxed for 6 h and monitored by TLC. After 6 h, there was still some starting material left in the reaction. The reaction mixture was evaporated by using rotary evaporator and the residue was subjected to silica gel column chromatography. The mobile phase was hexane (50 mL), then the mixture of hexane-ethyl acetate (7:3). The product was collected and the solvent was evaporated to obtain pale yellow solid (0.3831 g, 38.30% yield) with melting point of 117-118 ºC. UV λ_{max} 342 nm,; IR (KBr disc): 1704, 1618, 1609, 1489 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$) δ [ppm]: 1.31 (t, *J* = 7 Hz, 3H), 4.22 (q, *J* = 7 Hz, 2H), 5.93 (d, *J* = 15 Hz, 1H), 5.98(s, 2H), 6.69 (dd, *J* = 16, 11 Hz, 1H), 6.78 (d, *J* = 8 Hz, 1H), 6.81 (d, *J* = 15 Hz, 1H), 6.91(dd, *J* = 1.6, 9 Hz, 1H), 6.99(d, *J* = 1.6 Hz, 1H), 7.41 (dd, *J* = 10.5, 15.3 Hz, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): 167.1, 148.5, 148.2, 144.6, 140.1, 130.5, 124.5, 122.9, 120.4, 108.5, 105.8, 101.3, 60.2, 14.3 and MS (GC/MS): M^+ = 246**.**

Preparation of ethyl piperate by Steglich reaction [12] To a round bottom flask (50 mL) containing piperic acid (0.5003 g) in dry dichloromethane (15 mL), ethanol (1.0 mL), 4-dimethylaminopyridine (DMAP) (0.22 g) and dicyclohexylcarbodiimide (DCC) (0.57 g) were added. The mixture was stirred under nitrogen atmosphere for 24 h and the reaction was monitored by TLC. After the reaction was complete, the reaction mixture was evaporated by using rotary evaporator and the residue was subjected to silica gel column chromatography. The mobile phase was the mixture of hexane-ethyl acetate (7:3). The product was collected and the solvent was evaporated to obtain pale yellow solid (0.5227 g, 92.79% yield) with melting point of 117-118 ºC. UV λ_{max} 342 nm,; IR (KBr disc): 1704, 1618, 1609, 1489 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$) δ [ppm]: 1.31 (t, *J* = 7 Hz, 3H), 4.22 (q, *J* = 7 Hz, 2H), 5.93 (d, *J* = 15 Hz, 1H), 5.98(s, 2H), 6.69 (dd, *J* = 16, 11 Hz, 1H), 6.78 (d, *J* = 8 Hz, 1H), 6.81 (d, *J* = 15 Hz, 1H), 6.91(dd, *J* = 1.6, 9 Hz, 1H), 6.99(d, *J* = 1.6 Hz, 1H), 7.41 (dd, *J* = 10.5, 15.3 Hz, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): 167.1, 148.5, 148.2, 144.6, 140.1, 130.5, 124.5, 122.9, 120.4, 108.5, 105.8, 101.3, 60.2, 14.3 and MS (GC/MS): M^+ = 246

RESULTS AND DISCUSSION

The isolation of piperine from black pepper was uncomplicated process and the compound was obtained at a reasonable yield of almost 3%. For the preparation of piperic acid, alkali hydrolysis with potassium hydroxide gave enough material to go on to next step. The esterification of piperic acid with alcohol was performed by two different methods. The first method, which was the acid catalyzed esterification, used *p*-toluene sulfonic acid as a catalyst with Dean-Stark Apparatus to remove water from the reaction in order to push the reaction equilibrium to the product side. However, this reaction need to be refluxed in high temperature in toluene and the ethanol which was one of the reagents in the reaction could evaporate from the reaction during the process. The reaction was therefore not complete and the product was obtained at a poor yield. We then decided to use another reaction, the Steglich esterification, which could be done at room temperature (Figure 1).

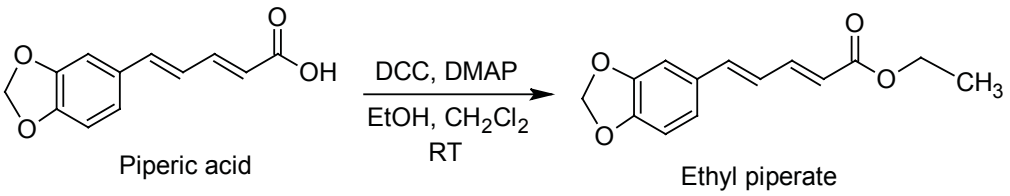

Figure 1 Steglich esterification reaction to prepare ethyl piperate

Steglich esterification reaction is a mild reaction which allows the conversion of sterically demanding and acid labile substrates. This reaction used dicyclohexylcarbodiimide (DCC) and 4-dimethyl-aminopyridine (DMAP) as activation agent and catalyst, respectively, to conduct more efficient esterification reaction than the classical one. It is better for works with the highly volatile compounds because the

reaction can be performed at room temperature. For the synthesis of ethyl piperate with Steglich reaction, we obtained ethyl piperate at a yield of 92.79% yield.

CONCLUSION

From our study, the isolation of piperine and the preparation of piperic acid derivatives are simple and efficient. Ethyl piperate is obtained through Steglich esterification reaction in a high yield. This preparation is in the first phase of projects to use ethyl piperate as UV protective agent, and also to apply ethyl piperate in liposomes form for drug delivery study.

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