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Isolation of Anti-malarial Active Compound from Yanang (*Tiliacora triandra* Diels)

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ABSTRACT

Malaria remains to be one of the serious problems in tropical countries because of the increase in antimalarial drug resistance. This investigation was to study the extraction of bioactive compounds with anti-malarial activity from Yanang (*Tiliacora triandra* Diels) root. The dried root was extracted with chloroform : methanol : ammonium hydroxide mixture (50:50:1). Further isolation and purification of the crude extract using column chromatography and crystallization techniques provided two pure alkaloid compounds: tiliacorinine (I) and tiliacorine (II) with 0.0082 and 0.0029 percent yield, respectively. Structures of I and II were confirmed by spectroscopy techniques and compared with reference data.

Key words: anti-malarial, Yanang, *Tiliacora triandra* Diels, tiliacorinine, tiliacorine

INTRODUCTION

Today malaria is found throughout the tropical and sub-tropical regions of the world and causes more than 300 million acute illnesses and at least one million deaths annually. Moreover, the increase of drug resistance of *Plasmodium falciparum* remains to be serious problems (WHO, 1998). Thailand is a resource of medicinal plants and many of them have claimed to be used as antimalarials. Hence, utilization of these plants has been considered.

Tiliacora triandra Diels, one of the medicinal plants, known in Thai as Yanang, belongs to Menispermaceae family. Its root has been widely used as antipyretic agent for all kinds of fever and also prescribed in the preparation of antimalarial in folk medicine (Fumio *et al*., 1990; Norman and Nuntavan, 1992). Tiliacorinine (I) and tiliacorine (II) which are bisbenzylisoquinoline alkaloids have been found and isolated by using a combination of chromatography and counter-current distribution in *Tiliacora* species, *Tiliacora racemosa* Colebr (Anjaneyulu *et al*., 1969) and their *in vitro* antimalarial activity against *Plasmodium falciparum* have been studied (Thaweephol *et al*., 1987). The isolation of tiliacorinine and tiliacorine from *T. triandra* roots by column of ion exchange resin; Amberlite IRA 400 has been reported (Thaweephol *et al*., 1974). Further preparative thin layer chromatography have more frequently used for purification of these two compounds (Pichaet and Boondate, 1981; Thaweephol *et al*., 1987). In this report we describe a simple column chromatographic method without further purification of the diastereomeric alkaloids, tiliacorinine and tiliacorine from *T. triandra* roots. Our method differs from those reported earlier with regard to stationary phase material, eluting solvent composition, and application.

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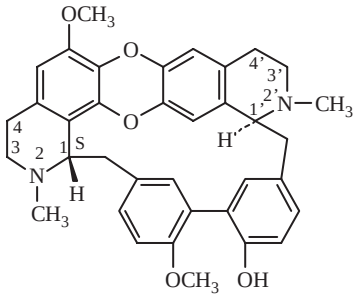
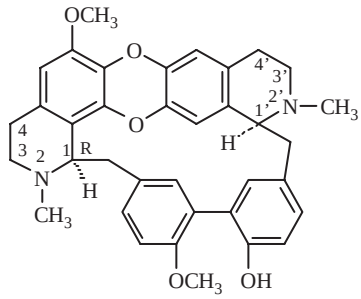


Tiliacorinine (I)



Tiliacorine (II)

MATERIALS AND METHODS

Chemicals

Silica gel 60 F_{254} size 0.040-0.0063 mm for column chromatography, thin layer chromatography (TLC) aluminium sheets 20 × 20 cm coated with silica gel 60 F_{254} and kieselguhr were obtained from Merck (Damstadt, Germany). Chloroform, methanol and ethyl acetate were commercial grade and were obtained from Rattana Trading Co. (Thailand). Analytical grade petroleum ether and heptane were obtained from SNP Co. (Thailand). Analytical grade ammonia was obtained from Carlo Erba (Thailand). Analytical grade ether was purchased from BDH Co. (Thailand). Mayer's reagent was prepared according to the reference method of USP 23.

Extraction of alkaloids from roots of T. triandra

The air-dried ground root of *T. triandra* was bought from Manora O-soad store, Phitsanulok, Thailand, dried in hot air oven at 70° C for 3 hours, milled to fine powder and then dried with the same procedure. The dried powder (2 kg) was macerated in chloroform : methanol : ammonia (50:50:1) at room temperature for 24 hours and filtered. The filtrate was evaporated under reduced pressure until dry. The residue from the filtration was further macerated following the same procedure until the portion of the filtrate gave a negative result with Mayer's test (USP 23). All evaporated extracts were combined and dissolved in small amount of chloroform. The chloroform layer was extracted in 10% sulfuric acid several times until the chloroform layer gave the negative result with Mayer's test. The combined aqueous layer was basified with dilute ammonia to precipitate water insoluble alkaloids. The mixed alkaloids were collected and dried in vacuum desiccator.

Isolation of tiliacorinine and tiliacorine

The mixed alkaloids (9.974 g) were dissolved in chloroform : methanol (1:1), and then filtered. The precipitate was discarded and the filtrate was evaporated under reduced pressure. The separation of active alkaloids was performed using column chromatography on a silica gel 60 F_{254} (4 × 40 cm) eluted with petroleum ether, heptane and finally with chloroform : methanol : ethyl acetate (5:1:3), with 30 ml fractions being collected as follows: fractions 1-34 (A), 35-41 (B), 42-52 (C), 53-69 (D), 70-81 (E), and 82-145 (F). The collected fractions were monitored using thin layer chromatography on silica gel GF_{254} having chloroform : methanol (9:1) as the solvent system. Collection B and D were evaporated and then crystallized in ether to give small pale yellow needles of tiliacorinine (165 mg) and tiliacorine (58 mg), respectively. Melting point measurement (Buchi 535, Japan), ultraviolet-visible (UV-Vis) spectrophotometry (Lamda20, Perkin Elmer,

Germany), infrared (IR) spectrophotometry (KBr pellet technique, Spectra 2000, Perkin Elmer, Germany), and proton nuclear magnetic resonance (^{1}H-NMR) spectrometry (Jeol JMN-A500 spectrometer, using tetramethylsilane as the internal standard) were used to identify the structures. Visualization of TLC was observed under ultraviolet light or by spraying with Mayer's reagent.

RESULTS AND DISCUSSION

The extraction of 2 kg grounded root of *T. triandra* gave 9.974 g total alkaloids. Further isolation provided two pure alkaloid compounds from collection B (165 mg, 0.0082%) and collection D (58 mg, 0.0029%). The UV spectra of compounds from collection B and D were identical at λ_{max} 290 nm. The IR spectra of these two compounds are shown in Table 1.

The ^{1}H-NMR spectra of collection B and D were in accordance with those of tiliacorinine and tiliacorine as reported by Guha *et al*. (1976). The ^{1}H-NMR spectrum in CD_3Cl of collection B (Figure 1) showed the 2′-NCH_3 resonance at lower field (δ2.65, s, 3H) and the 2-NCH_3 at higher field (δ2.32, s, 3H). The signals of two OCH_3 appeared at 3.87 (s, 3H) and 3.99 (s, 3H). ^{1}H-NMR spectrum in CD_3Cl of collection D (Figure 2) showed the 2′-NCH_3 resonance at lower field (δ2.64, s, 3H) and the 2-NCH_3 gave rise to a signal at higher field (δ2.34, s, 3H). The signals of two OCH_3 appeared at 3.87 (s, 3H) and 3.93 (s, 3H). Two of these compounds, from collection B and D also had six protons representing the two sets of methylene bridge protons (δ2.82, m, 3H, and δ3.00, m, 3H), the two methine neighbors situated on the two heterocyclic rings and nine aromatic protons.

The UV-Vis, IR and ^{1}H-NMR spectra of compound from collection B were slightly distinguishable from that of collection D. These results confirmed that the compounds of both samples were either tiliacorinine or tiliacorine. However, comparing the melting points of these compounds (Table 2) to those reported by Thaweephol *et al*. (1987), it could be concluded that collection B (R_f 0.73) was tiliacorinine and collection D (R_f 0.57) was tiliacorine.

CONCLUSION

Tiliacorinine and tiliacorine, diastereomeric compounds from the roots of *T. triandra* were completely separated by using simple column chromatography and crystallization techniques. This method has never been used with this plant before. The result provided high percentage yield of tiliacorinine and tiliacorine that could be used for structure modification and determine the antimalarial activity relationship. The designing and modifying of tiliacorine and tiliacorinine structures are in progress to increase antimalarial activity for further study.

Table 1 IR spectra bands of compounds from collection B and D.

The bands	Frequency (cm^{-1})	
	Collection B	Collection D
O-H stretching	3,394	3,402
C-H stretching of aromatic moieties	1,587 and 1,502	1,587 and 1,498
C-O stretching	1,277-1,122	1,273-1,118

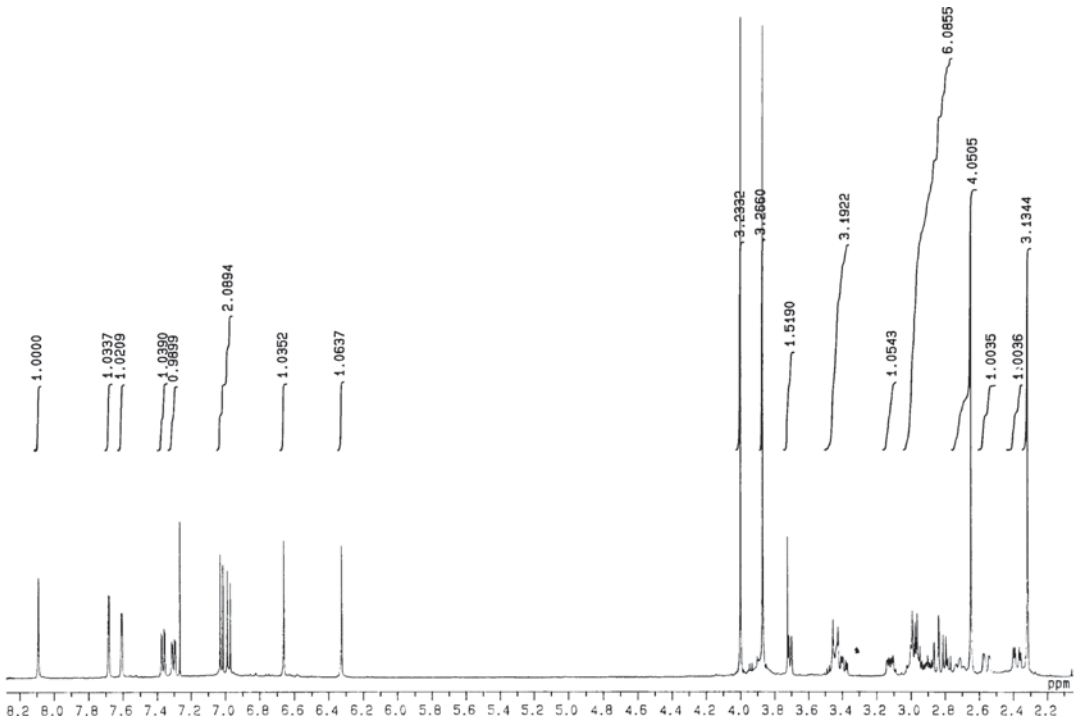


Figure 1 The ^{1}H-NMR spectrum of collection B in CD_3Cl.

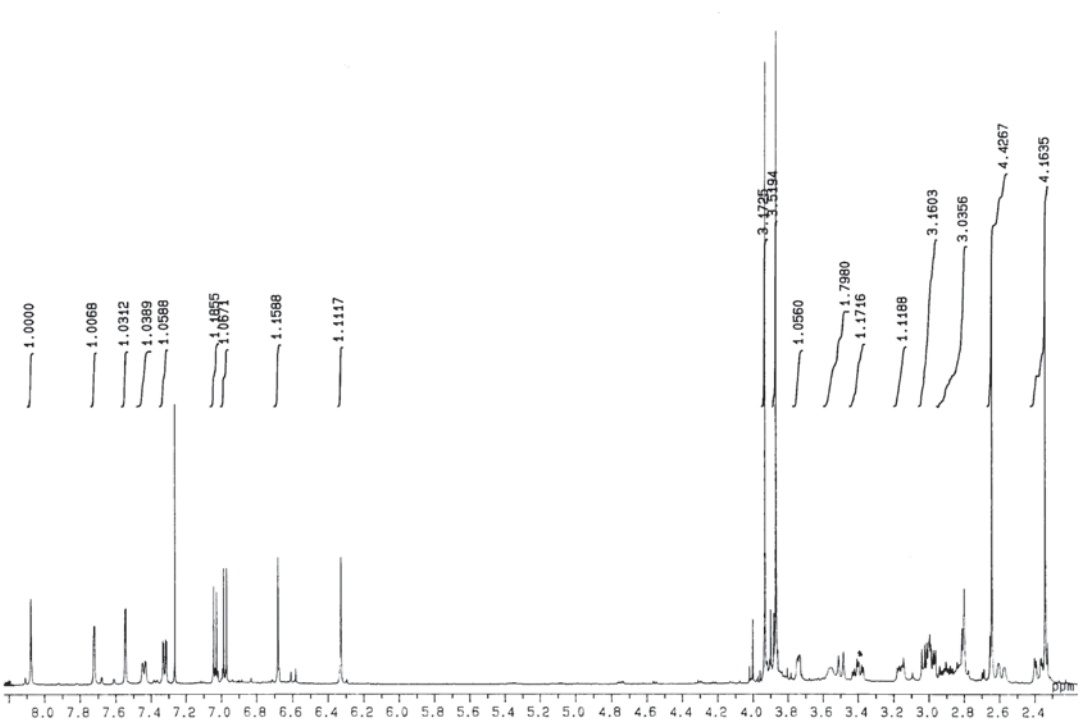


Figure 2 The ^{1}H-NMR spectrum of collection D in CD_3Cl.

Table 2 Melting point of tiliacorinine and tiliacorine.

Compounds	Melting point (°C)	
	Thaweephol *et al*.	Our result
Tiliacorinine	162-175	167-170 [a]
Tiliacorine	264-267	262-264 [b]

[a] compound crystallized from collection B
[b] compound crystallized from collection D

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